

# Effects of Urbanization on Disease Prevalence in Managed Honey Bee Hives in Hamilton County, TN

Caitlin Jarvis and DeAnna E. Beasley



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Cover Photograph: Photo of *Varroa destructor* on *Apis mellifera* in Hamilton County, Tennessee taken on April 13th 2021. Photograph by Joshua Crow.

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# Effects of Urbanization on Disease Prevalence in Managed Honey Bee Hives in Hamilton County, TN

Caitlin Jarvis<sup>1\*</sup> and DeAnna E. Beasley<sup>1</sup>

**Abstract** - Expanding urban development in the Southeastern United States may increase disease exposure on economically essential organisms, including Western Honey Bees. The Western Honey Bee is a generalist pollinator, capable of pollinating many crops, native plants, and ornamental flowers. The honey bee is the most commonly recorded pollinator in the world. We surveyed 18 apiaries containing a total of 53 hives for diseases, pests and colony size from 2021–2022 in Hamilton County, Tennessee at varying levels of urbanization. Pests and pathogens analyzed included Varroa Mites and Small Hive Beetles. Parasite levels were analyzed at 1.0 km intervals from 0.5–9.5 km radii from each hive. Varroa Mite levels significantly decreased with increased urbanization, conversely numbers increased with urbanization. There was a weak negative correlation between Varroa Mite load and the likelihood of colony survival and a weak positive correlation of abundance and survival. These results are significant as it indicates a changing disease ecology based on the degree of urbanization. As both urbanization and the demand for pollination services increase, it is vital to understand how Western Honey Bees will adapt to changing environments and pressures associated with urbanization.

## Introduction

Urbanization in the Southeastern United States (U.S.) is projected to grow rapidly in coming decades, with a doubling to tripling of urbanized lands expected (Terando et al. 2014). Land will likely be developed at a greater rate than human population growth, resulting in decreased population densities over larger areas of the landscape. This urban sprawl will increase habitat fragmentation as roads and suburbs increase outside of metropolitan areas (Bounoua et al. 2018, Terando et al. 2014). Intensive management of flowering species by gardeners can increase the floral resources available to pollinators, including blooming outside the timeframe of native flowers (Fitch et al. 2019, Wilson and Jamieson 2019). Urban environments provide patchy, concentrated resources for wildlife. This theoretically increases the potential for disease transfer as wildlife are forced to forage in concentrated areas (Bradley and Altizer 2007).

Urban development has many effects on the landscape including increased temperatures, elevated CO<sub>2</sub> levels, and drier conditions when compared to surrounding natural habitats. These transitions are similar to those expected by global climate change (Lahr et al. 2018). Studying the effects of urbanized environments on wildlife and managed species can thus be used to predict global shifts due to climate change. As an example, cities experience increased temperatures compared to surrounding habitat. This leads to phenological shifts such as earlier blooming for some flowering plants, or shifts in the breeding season of insects (Lahr et al. 2018). This has been observed with bees and apple trees in the UK (Wyver et al. 2023). Such shifts may be beneficial through providing additional foraging opportunities during nectar dearths, negative by causing insect breeding cycles to miss optimum forage, or neutral with little observable effect (Fitch et al. 2019, Lahr et al. 2018, McCune et

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al. 2020, Wilson and Jamieson 2019). Various pollinating bees (including honey bees) have been found to be more active on some floral resources in urban areas than in rural areas, especially when the flowering period was artificially delayed or advanced. This trend was not seen in all observed pollinators (Zaninotto et al. 2020).

Altered community composition is a common response to urbanization (Diamond et al. 2015). Many studies have shown worldwide declines in insects, including declines in native and managed hymenopterans (Cameron et al. 2011, Sánchez-Bayo and Wyckhuys 2019). This, however, depends upon the species. Cavity-nesting, large-bodied, and long-tongued bee species tend to be positively affected by urban environments when compared to smaller, ground-nesting species (Baldock 2020, Belsky and Neelendra 2019, Wilson and Jamieson 2019). Generalist species also tend to adapt to anthropogenic stressors more readily than specialist species (Belsky and Neelendra 2019, Diamond et al. 2015). Fitch et al. found an increase in wild exotic bee species abundance and richness in more urbanized areas. This was seen in cavity-nesting native bees, though the increase was not as strong. This trend was not seen with non-cavity nesting native bees (Fitch et al. 2019).

Pollinators are widely recognized as critical to human food supplies and biodiversity. Roughly 35% of human crops are dependent to some extent upon animal pollination (Klein et al. 2007). In recent decades, declines in pollinators have been observed, while human demand for pollination services has increased (Belsky and Neelendra 2019, Koh et al. 2016). *Apis mellifera* L. (Western Honey Bee, hereafter Honey Bee) is a generalist pollinator capable of pollinating a wide range of native and agricultural plants; and worldwide, is the most commonly recorded insect pollinator (Hung et al. 2018, Klein et al. 2007). Since 2010 there has been an estimated annual Honey Bee colony loss in the United States of 39.7% (Steinhauer et al. 2021). Since beekeepers order new bees and split colonies, much of this is recovered annually, bringing the overall loss rate to 0.9% (Sánchez-Bayo and Wyckhuys 2019). However, these approaches are not sustainable in the long term due to increased cost, workload, and a reduction in economic demand for domestic honey over the years (Le Conte and Navajas 2008, Sánchez-Bayo and Wyckhuys 2019, Smith et al. 2013).

As a managed species, Honey Bees may be kept at artificially high densities (Egerer and Kowarik 2020). The combination of high hive densities and concentrated foraging patches would thus theoretically aid in greater pathogen transfer. Youngsteadt et al. (2015) found increased pathogen intensity of *Nosema* and black queen cell virus in Honey Bees in more urbanized areas. Conversely, Appler et al. (2015) did not find a significant effect of urbanization on immune responses. This indicates a need to understand the effects of urbanization more fully on pollinating insects.

Our study aimed to determine the effects of urbanization on the health and colony size of managed Honey Bee hives in and around Hamilton County, Tennessee. For this study, we had 2 hypotheses. 1) Urbanization would increase the observed disease levels in Honey Bee hives, as bees would be forced to forage in more concentrated areas. This would also expose bees to increased urbanization stressors such as increased temperatures, drier conditions, and increased CO<sub>2</sub> levels. 2) Hives with higher disease levels would have a lower survival rate, due to a combination of these stressors and pathogen pressure.

## Materials and Methods

### Study Species

The Honey Bee is a member of the family Apidae in the order Hymenoptera. The Honey Bee is native to Europe, Africa, and the Middle East, but has been spread nearly worldwide

for honey production and pollination, where it has adapted to habitats that have sufficient floral resources, in both managed and feral colonies (Martin et al. 1980). Honey Bees were first brought to the United States in the early 1600s, and by 1800 they were widely distributed from the Atlantic coast to the Mississippi River (Martin et al. 1980). In 2020, approximately 148 million pounds of honey were produced in the United States from 2.71 million colonies, generating over 300 million dollars of revenue (National Agricultural Statistics Service 2021). In more recent years, pollination services have become increasingly profitable. Total animal pollination values in the United States attributed to Honey Bees reached 11.68 billion in 2009 (Calderone 2012). Travel distances of foraging Honey Bees are variable, from less than 0.5 km to the extreme of 13.5 km, depending on the surrounding landscape, season, and particular resource (Beekman and Ratnieks 2000, Couvillon et al. 2015, Eckert 1933, Steffan-Dewenter and Kuhn 2003). Honey Bees will typically forage greater distances in the summer months when compared to spring and autumn. Overall, a radius of 1.5 km can be considered average (Youngsteadt et al. 2015).

*Varroa destructor* Anderson and Trueman (Varroa Mite), introduced from Asia where it has long parasitized *Apis cerana* Fabricius (Asian Honey Bee), has now spread nearly worldwide and is found in virtually every *A. mellifera* colony in the United States. Varroa Mites feed on the fat bodies of Honey Bees which reduces fitness and immune responses, and act as vectors for several viruses. Varroa is widely accepted as one of the most serious pests of Honey Bees and plays a significant role in colony losses (Genersch 2010, Gregorc and Sampson 2019, Ramsey et al. 2019, Rosenkranz et al. 2010). Mites reproduce in Honey Bee brood cells and are carried on bees throughout the hive and to new hives. Varroa Mites are not able to transfer to new hives without Honey Bees or beekeeping equipment (Genersch 2010). The “acceptable threshold” for Varroa infestation varies with location, viruses carried, and the colony itself, but is typically recommended around 2–3% (Giacobino A et al. 2016, Gregorc and Sampson 2019).

Deformed wing virus (DWV) is so named because of the classic symptoms of wrinkled, deformed wings on heavily infected bees. Symptomatic DWV in Honey Bees occurs with high viral loads vectored by Varroa, yet is largely covert when mites are absent. Thus, the presence of Varroa is a critical factor in DWV pathogenicity in Honey Bees; the presence of symptomatic DWV is often associated with a collapsing colony (de Miranda and Genersch 2010, Genersch 2010). Symptomatic DWV has been found in commercial and wild *Bombus* spp. species, where exposure likely came from Honey Bees (Fürst et al. 2014, Genersch et al. 2006).

*Aethina tumida* Murray (Small Hive Beetles) were first observed in the United States in 1998, where they have since become a significant cause of colony and honey loss. Honey Bees are often able to prevent beetle infestation from reaching damaging levels. However, even strong colonies may be lost to larger invasions (Cuthbertson et al. 2013, Hood 2004). Honey Bees have been shown to build propolis prisons with guard bees to trap adult beetles. Bees have also been shown to remove beetle eggs from capped bee brood (Hood 2004). Adult and larval beetles prey upon the brood and eggs of Honey Bees. A combination of laying eggs and defecation from adult beetles will cause honey to ferment and slide out of frames, becoming unsuitable for bee or human consumption (Hood 2004).

## Study Site

Most of the study was conducted in Hamilton County, Tennessee; one hive was in bordering Catoosa County, Georgia (Fig. 1). Hamilton County is located at 34.98298, -85.47572 and 35.45641, -85.12680 and has an area of 1,490km<sup>2</sup> with an elevational range



of 192 m to 724m above sea level. Land use types include forest, agricultural land, and developed land (Dewitz and U.S. Geological Survey 2021). Climate is humid subtropical, but can experience excessive rainfall, drought, and abnormal temperatures (Terando et al. 2018). As of 2021 Hamilton County has 1,828 registered hives in 316 apiaries (Fig. 2) (Tennessee Department of Agriculture 2021). An invitation to participate was sent to all registered beekeepers in Hamilton County. Participants in Tennessee were required to be registered with their state apiary inspector or be in the process of registering (Tennessee Apiary Act 1995).

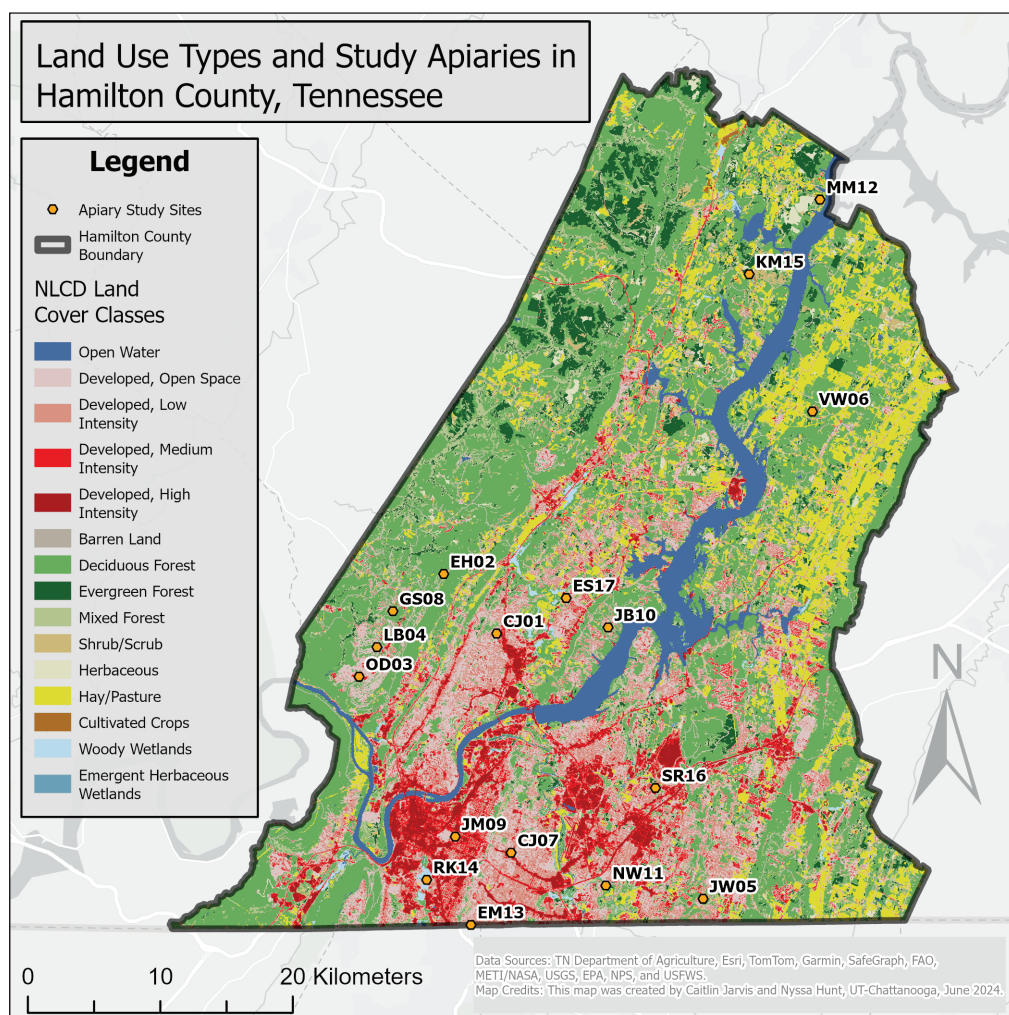


Figure 1. Study apiaries (n=17) and land use types in Hamilton County, TN. Most of the land is forested (47.74%). 31.20% of land is developed. *Open Water* 5.73%; *Developed, Open* 12.79%; *Developed, Low* 2.81%; *Developed, Medium* 10.29%; *Developed, High* 5.31%; *Barren Land* 0.16%; *Deciduous Forest* 36.12%; *Evergreen Forest* 5.18%; *Mixed Forest* 6.44%; *Shrub-Scrub* 0.95%; *Herbaceous/Grassland* 1.34%; *Pasture/Hay* 12.23%; *Cultivated Crops* 0.05%; *Woody Wetland* 0.48%; *Emergent Wetland* 0.11%. One additional apiary with 6 hives was located in Catoosa County, GA.

### Disease and pest survey

Surveys of 53 selected hives were conducted once each in the summer and fall of 2021, and in the spring of 2022. Hives were inspected a minimum of once to a maximum of 3 times in the study. Hives were opened between 10.0° C – 37.7° C when there was no precipitation, in order to minimize negative impacts to the hive. Diseases surveyed for included American foulbrood, European foulbrood, chalkbrood, sacbrood virus, and deformed wing virus. Parasites surveyed included Varroa, Small Hive Beetles, and Wax Moths. On some occasions beekeepers would opt out of Varroa surveys due to concern over the health of the colony, inclement weather, or time constraints. In total 59 Varroa surveys were performed.

One common way to check for Varroa is with the “sugar shake” method (Dietemann et al. 2013). We selected a frame of bees with uncapped brood. These frames contain more nurse bees, which are shown to be more attractive to mites (Peck and Seeley 2019, Xie et al. 2016). The frame was shaken into a bucket, then roughly 1/2 cup, or 300 bees, were transferred to a mason jar with a screen lid. Powdered sugar was then added, and the jar was shaken for 1 minute to coat the bees. After resting for 2 minutes, the jar was shaken upside down to dislodge mites for

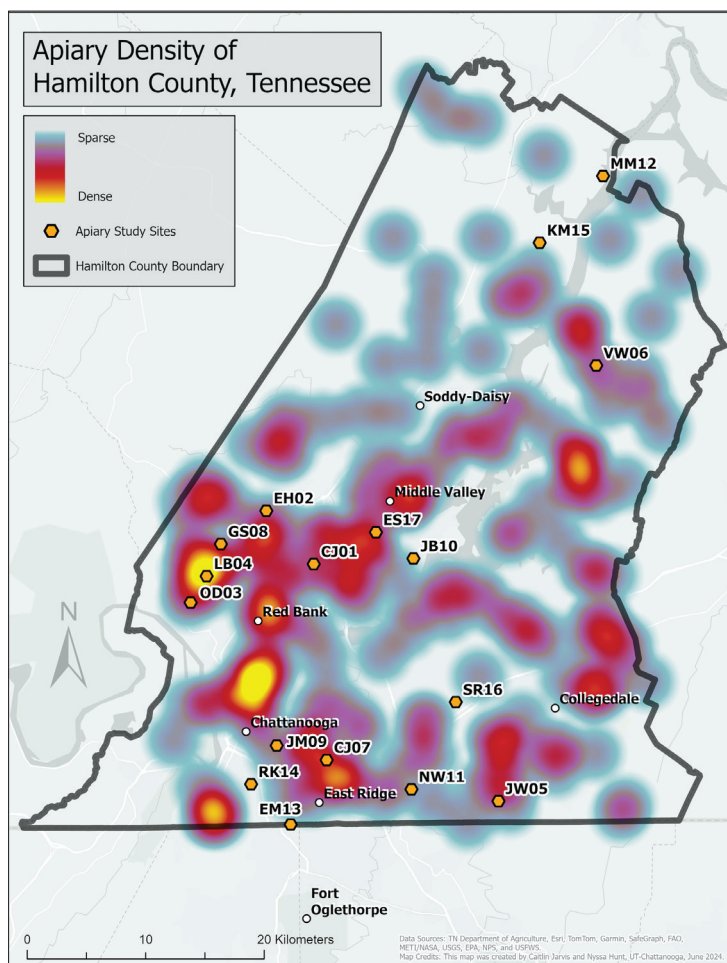


Figure 2. Density of all registered apiaries in Hamilton County, TN.

counting. The number of mites per 300 bees was used to estimate Varroa load. B  k et al. found no significant difference between this method and freezing and flotation in a lab, with 93% of mites found in the sugar shake method (B  k et al. 2009). Alternative methods of surveying for Varroa requires sacrificing bees, which most beekeepers were not willing to do. Overall, only Varroa and Small Hive Beetles were present in sufficient quantities for analysis.

Small Hive Beetles were surveyed by counting of individual beetles on frames and on the sides of hives in the field. Wax moths and cases of DWV were also counted. The diseases of brood were surveyed by visual observation of frames.

### Colony size estimates

The number of bees in a hive can be estimated from the area of a frame that is covered by bees. According to the COLOSS Bee Book, there are approximately 1.38 bees/cm<sup>2</sup> on a standard Langstroth frame (Delaplane et al. 2012). By outlining the area of a frame covered by bees in ImageJ, we were able to determine the number of bees within a hive (Fig. 3).

### Landscape type

Landscape type maps were obtained from the Multi-Resolution Land Characteristics (MRLC) Consortium (Dewitz and U.S. Geological Survey 2021). ArcGIS Pro 3.0 was used for mapping. Youngsteadt et al. analyzed the percentage of impervious surfaces within radii of 0.1 km to 3.0 km. We used a similar method, but expanded the range and used the percentage of urbanization within potential foraging radii from 0.5 km to 9.5 km at 1.0 km intervals (Fig. 4) (Youngsteadt et al. 2015). In the literature 9.5 km is the maximum foraging distance reported in a landscape similar to that of Hamilton County (Beekman and Ratnieks 2000). The percentage of urbanized land within these foraging distances ranged from 4.45–99.90%. Urbanized land was determined from developed land and contained low to high development.

### Statistical analysis

R was used for analysis. Only Varroa and Small Hive Beetles were present in sufficient quantities for analysis ( $n = 59$  for Varroa and  $n = 86$  for Small Hive Beetles). For Varroa counts, 6 outliers were identified by a boxplot. After reviewing data, we determined that these outliers were natural variation rather than measurement error, so they were included in analysis. For Small Hive Beetles, 14 outliers were removed due to potential measurement error. A mixed generalized linear model with a Gaussian distribution was run to assess parasite levels (Varroa counts and Small Hive Beetle counts respectively) as a function of colony size and urbanization at 10 different foraging radii (0.5 to 9.5 km at 1 km intervals) using the nlme package and lme() function for each season. Hive was included as the random effect. AIC selection was used to select the best fit model for the data using the aictab () command.

To determine which factors influenced survival, we used a mixed generalized linear model with a Poisson distribution to assess survival as a function of colony size, urbanization within the 10 different foraging radii, and disease levels with hive as a random effect.

## Results

Due to colony death and beekeeper drop out, each season has progressively fewer participants. During the summer of 2021 there were 44 hives, during fall 39 hives, and during spring 2022 there were 16 hives. One participant was added in fall, along with 3 hives from a previously used apiary. Overall colony loss (not including those who did not respond to the end of the survey) was 58.14%.



A trend of lower Varroa levels in more urbanized areas was observed. This trend continued through radii across seasons, but the strength of this relationship varied. Overall, there were 59 Varroa surveys, 32 in summer 2021, 23 in fall 2021, and 4 in spring 2022. Spring was not included as its own season in analysis due to the low sample size (however spring data are available in the combined seasons model). An AIC estimator was used to compare urbanization within foraging radii to determine which model was the best fit per season. For all seasons combined this was 1.5 km<sup>2</sup>. At this radius there is a significant negative correlation of urbanization and Varroa load ( $n = 59$ ,  $p = 0.0195$ ,  $r = -0.3020$ , Fig. 5). During summer 2021 the AIC estimator selected 1.5km<sup>2</sup> as the best fit. At this radius there is a significant negative correlation of urbanization and Varroa load ( $n = 32$ ,  $p = 0.0318$ ,  $r = -0.3801$ , Fig. 6). During fall 1.5km<sup>2</sup> was no longer considered the best fit model but was moderately significant and  $p=0.0550$ . The AIC estimator selected 9.5km<sup>2</sup> as the best fit model during fall 2021 ( $n = 23$ ,  $p = 0.03361$ ,  $r = -0.4444$ , Fig. 7).

There was a positive relationship between urbanization and Small Hive Beetle count when all seasons were combined. This relationship was seen at all foraging radii, but the strength of the relationship varied. Outliers ( $n = 13$ ) were identified with a boxplot and removed. Across all seasons combined the AIC model showed 9.5 km as the best fit ( $n = 72$ ,  $p = 0.0025$ ,  $r = 0.3758$ , Fig. 8). During the summer of 2021 the AIC showed 9.5 km as the best fit ( $n = 40$ ,  $p = 0.0009$ ,  $r = 0.5064$ , Fig. 9). During the fall of 2021, the AIC selected model was 0.5 km ( $n = 21$ ,  $p = 0.1032$ ,  $r = 0.3656$ , Fig. 10). During spring 2022 the AIC model showed 9.5 km as the best fit ( $n = 11$ ,  $p = 0.2029$ ,  $r = 0.4162$ , Fig.11).

### Survival and colony size

The size of a colony had a negative correlation with the likelihood of surviving the study with larger colonies less likely to survive ( $n = 100$ ,  $rpbi = -0.4945$ ). There was a negative correlation between higher Varroa loads and the likelihood of surviving the study ( $n = 53$ ,  $rpbi = -0.2370$ ). There was a positive correlation between Small Hive Beetle count and

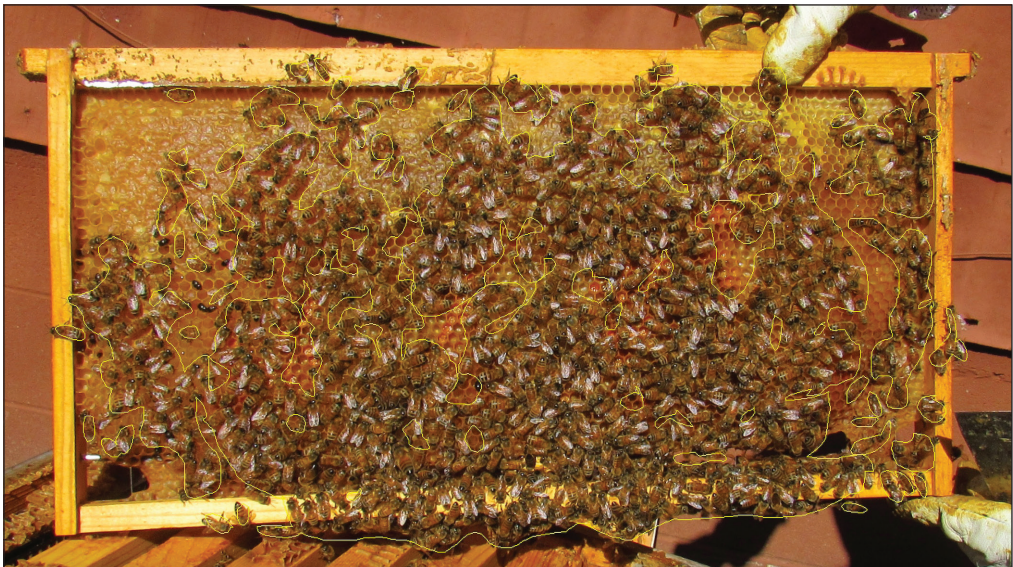


Figure 3. Example of productivity measurement in ImageJ. One typical frame with an area of 698.007cm<sup>2</sup> Shows ~ 963.250 bees when multiplied by the standard 1.380 bees/cm<sup>2</sup>.

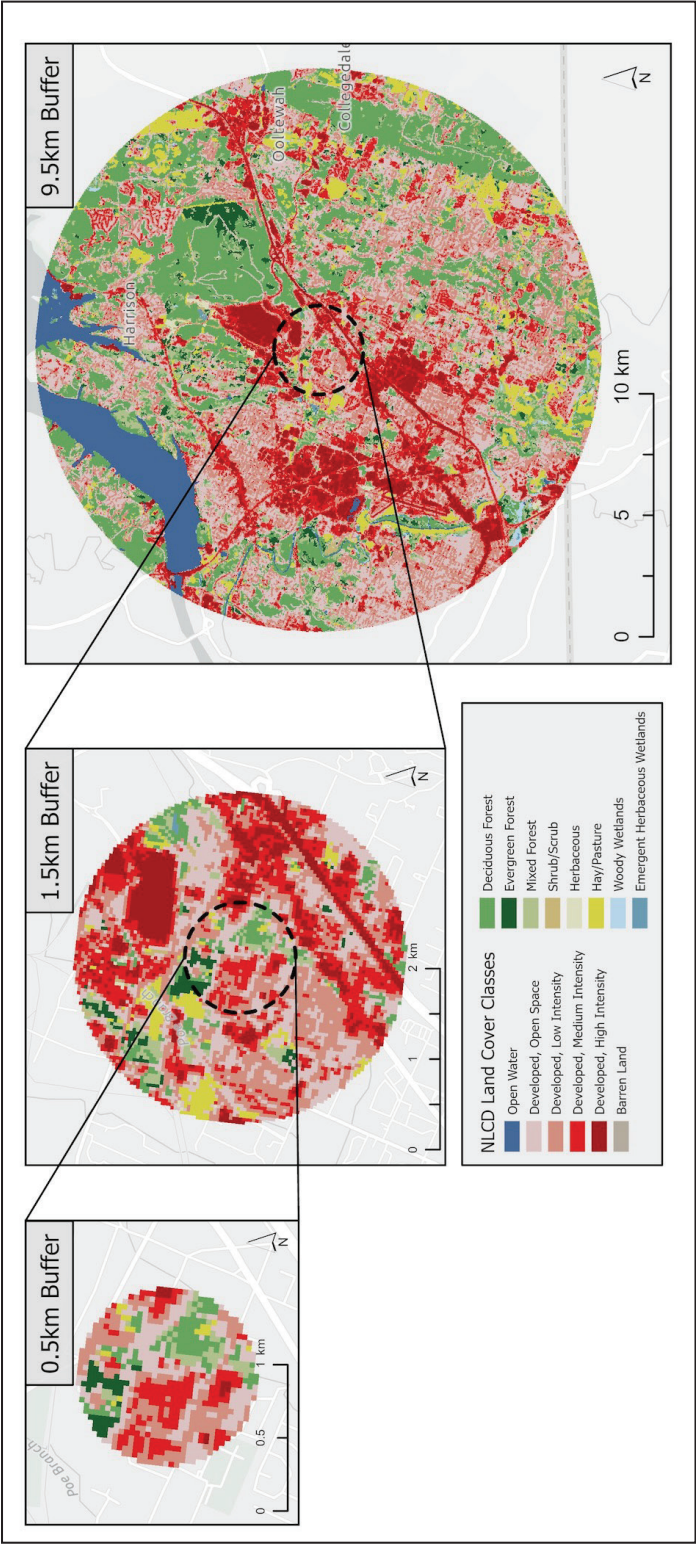


Figure 4. The minimum (0.5 km), average (1.5 km), and maximum (9.5 km) foraging radii of the honey bee around apiary SR16. Within 0.5 km 75% of land is urbanized, at 1.5 km 84% of land is urbanized, and at 9.5 km 58% of land is urbanized.

survival ( $n = 72$ ,  $rpbi = 0.1312$ ). There was no significant correlation at any radius between urbanization and the likelihood of surviving the study.

## Discussion

Across all seasons, the level of Varroa infestation was negatively correlated with higher levels of urbanization. This trend continued through other foraging radii across seasons, but the strength of this relationship varied. The AIC selected model for all seasons combined was 1.5 km. A summary can be found in Figures 5–7. These results were unexpected as concentrated resources found in urbanized areas theoretically would aid in

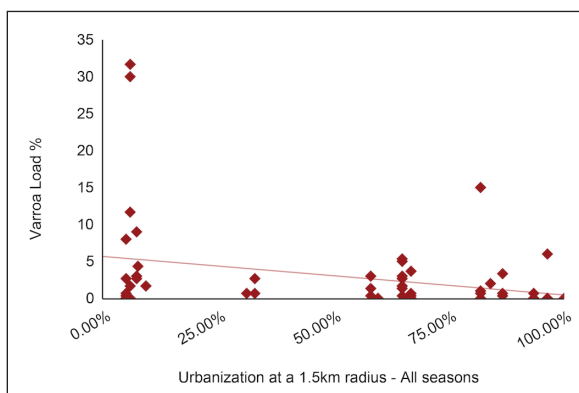


Figure 5. Varroa load by the percentage of urbanized land within a 1.5km radius from hives, all seasons combined. ( $n = 59$ ,  $p = 0.0195$ ,  $r = -0.3020$ ).

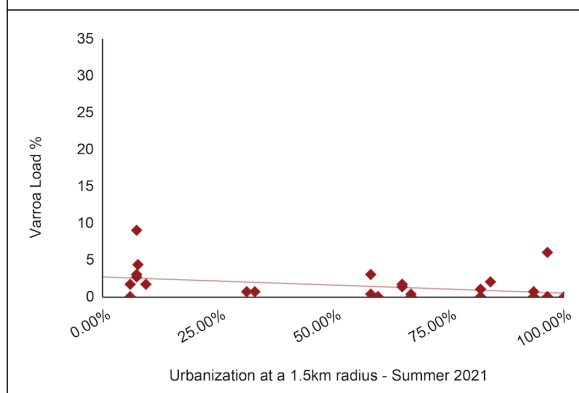


Figure 6. Varroa load by the percentage of urbanized land within a 1.5km radius from hives during summer 2021. ( $n = 32$ ,  $p = 0.0318$ ,  $r = -0.3801$ ).

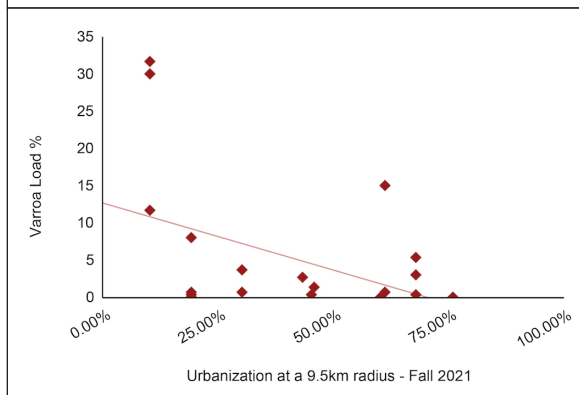


Figure 7. Varroa load by the percentage of urbanized within a 9.5 km radius from hives during fall 2021. ( $n = 23$ ,  $p = 0.03361$ ,  $r = -0.4444$ ).

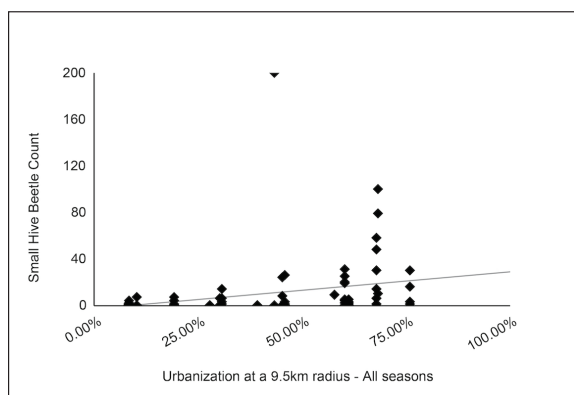


Figure 8. Small Hive Beetle count by the percentage of urbanization at a 9.5 km radius from hives, all seasons combined. ( $n = 72$ ,  $p = 0.0025$ ,  $r = 0.3758$ ).

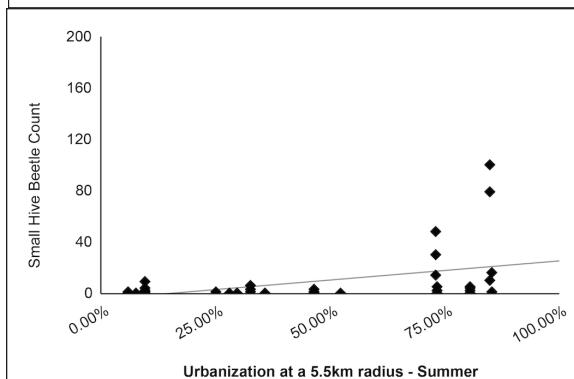


Figure 9. Small Hive Beetle count by the percentage of urbanization at a 9.5 km radius from hives during summer 2021. ( $n = 40$ ,  $p = 0.0009$ ,  $r = 0.5064$ ).

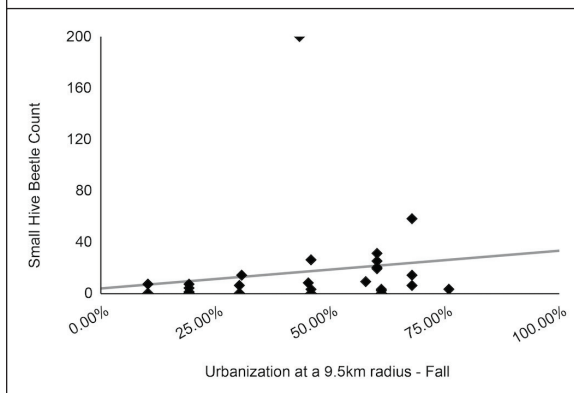


Figure 10. Small Hive Beetle count by the percentage of urbanization at a 0.5 km radius from hives during fall 2021. ( $n = 21$ ,  $p = 0.1032$ ,  $r = 0.3656$ ).

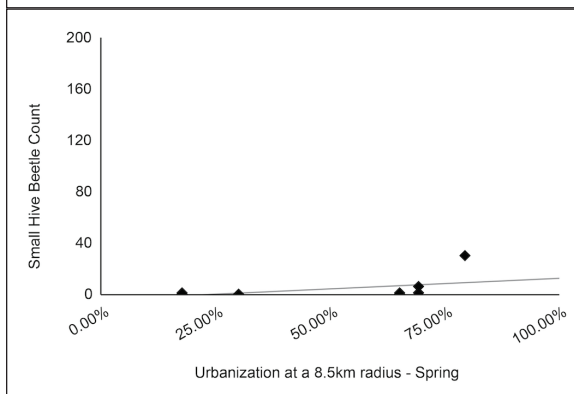


Figure 11. Small Hive Beetle count by the percentage of urbanization at a 9.5 km radius from hives during spring 2022. ( $n = 11$ ,  $p = 0.2029$ ,  $r = 0.4162$ ).



higher levels of parasite transfer (Bradley and Altizer 2007, Egerer and Kowarik 2020). Youngsteadt found increased pathogen intensity of *Nosema* and black queen cell virus in Honey Bees in more urbanized areas (Youngsteadt et al. 2015). There is a lack of previous research focusing on Varroa levels and how that may be influenced by specific environments, including across urban-rural gradients. Further research on environmental effects on Varroa levels is warranted.

As an exotic and cavity-nesting species, Honey Bees may benefit from urban environments. Urban environments have maintained gardens that can offer nectar resources outside the natural bloom period (Baldock 2020, Belsky and Neelendra 2019, Wilson and Jamieson 2019). As a generalist, Honey Bees are generally well equipped to take advantage of exotic plants. Although all hives in this study were managed, it remains possible that there was less competition from ground-nesting native bees and feral Honey Bees in more urban areas that could have increased fitness of Honey Bees (Belsky and Neelendra 2019, Fitch et al. 2019).

Notably, during Varroa surveys, 2 hives from the same apiary had exceptionally high Varroa loads of 31.76% and 30.0% and exhibited symptomatic deformed wing virus during the summer of 2021. Both hives were still alive during the final survey. This was far above the typical recommended treatment threshold of 2–3%. Differences in the pathology of DWV may be explained by different strains, which have only recently been identified (Posada-Florez et al. 2019). Small-scale beekeepers have been known to say that once a hive is showing visible DWV it is beyond the point where the colony can recover. Indeed, DWV presence is often accepted as a later stage of colony death (de Miranda and Genersch 2010, Genersch 2010). These results suggest that unavoidable colony death is not necessarily the case, and treatment can still enable the colony to recover. Further research into pathology of DWV should focus on strains of the pathogen.

There was a positive relationship between urbanization and Small Hive Beetle totals when all seasons were combined. This relationship was seen at all foraging radii, but the strength of the relationship varied. The AIC model showed 9.5 km as the best fit for all seasons combined. A summary can be found in Figures 8–11. While 9.5 km is within the known foraging radius of the Honey Bee, it is on the extreme side of what is possible (Beekman and Ratnieks 2000). Overall, this was in line with the expected results of greater pathogen presence in more urbanized areas. Previous studies have shown that Small Hive Beetles are able to travel considerable distances with swarming Honey Bees, and may be attracted to stressed colonies (Spiewok et al. 2008). Thus, they are able to travel to colonies with or without Honey Bees. This may give them a dispersal advantage over fully phoretic Varroa Mites. In turn, Small Hive Beetles may be able to overcome stressors of urban environments to reach new colonies.

Larger colonies were less likely to survive until the end of the study. This may be explained by larger colonies tending to have higher Varroa loads, as the Varroa load increases rapidly as the colony grows and then downsizes before winter. Frequent swarming and splits have been proposed as a way to keep colonies smaller and reduce Varroa buildup (van Alphen and Fernhout 2020). However, the 2 hives with the highest Varroa loads survived until the end of the study. There may be further factors to explain this observation. The genetics of Honey Bees may be important for resistance to Varroa Mites (O'Shea-Wheller et al. 2022). We did not have access to information about the strains or genetics of Honey Bees in this study.

There was no significant correlation at any radius directly between urbanization and the likelihood of surviving the study. There was a negative correlation of higher Varroa

loads and likelihood of survival. Conversely, there was a positive correlation of Small Hive Beetle abundance and likelihood of surviving the study. Both of these relationships were weak, however. Further study should be conducted to investigate these trends.

Other studies on urbanization have yielded mixed results. While using colony weight as a predictor of winter survival, urbanized and forested land were less valuable to Honey Bees than agricultural and herbaceous land in Canada. (Richardson et al. 2023). Disease levels of *Nosema* spp. and black queen cell virus were found to be greater in more urbanized areas (Youngsteadt et al. 2015). Conversely, a study by Samuelson found lower levels of *Nosema* spp. in more urbanized areas along with a greater colony strength (measured by bee-covered frames). One potential explanation is a greater presence of *Nosema* spp. in the environment due to a greater number of commercial bumblebees (Samuelson et al. 2020). These conflicting results indicate a need for further research. The overall colony loss rate from our study was 58.14%, above the national average of 39.7%.

Phenological shifts of pollinators and their plants will be of critical importance to undeveloped and developed lands alike. Cities may be useful as predictors of global climate change, due to similar effects of urbanization and changing climates (Lahr et al. 2018). Varroa mites have been a major cause of Honey Bee declines in many parts of the world. To our knowledge, there have been no other studies investigating Varroa load across an urban-rural gradient, and no studies investigating the relationship of Varroa load and Climate Change. Thus, further investigation of these phenomena is warranted.

The main limitation for this study was reduced amount of continued involvement by volunteer participants. By the end of the study several participants had dropped out of the study. Some of these were due to loss of colonies, others were due to an end of communication. Increasing the initial number of participants in future studies can assist in retaining a robust number participants. Even under ideal conditions loss of Honey Bee colonies can be significant. Future studies should try to seek more participants than is needed to ensure adequate numbers at the end.

Landscape use type was incorporated into this study. However, we found a lack of studies related to elevation in relation to Honey Bee foraging. In areas such as the southeastern United States there can be a significant variation in elevation. This study covered elevations from 201 to 599 m above sea level. An exploration of elevational effects of Honey Bee foraging and disease effects is an ideal next step in research.

## Conclusion

The disease ecology of the Honey Bee may change based on the degree of urbanization. Previous studies have shown pathogen pressure to increase with urbanization, or be unaffected (Youngsteadt et al. 2015). The observation that Varroa presence might be lower in urbanized areas is significant, as mites are one of the most important pathogens affecting Honey Bee colonies. Further research is warranted to explore the relationship of urbanization and pathogen pressures on Honey Bees. Cooperation with national loss and disease surveys will aid in making information more widely available to the public.

As both urbanization and the demand for pollination services increase, it is vital to understand how Honey Bees will adapt to changing environments and pressures. Honey Bees are the most commonly recorded pollinator in the world (Hung et al. 2018, Klein et al. 2007). They are not always a substitute for native pollinators (Belsky and Neelendra 2019, Winfree et al. 2007), however they contribute billions to ecosystem services (Jordan et al. 2021). As a generalist, the Honey Bee is able to pollinate a wide variety of plants (Klein et

al. 2007). Thus, the Honey Bee is vital to human crop production and to pollinating wild plants in many instances where native pollinators are absent. As Honey Bees are relatively large and easy to observe compared to native pollinators, they can be useful in monitoring for trends in general pollinators in some instances (Heard et al. 2017).

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