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Article

Nature's reset: The effect of native and invasive plant forage on honey bee nutrition and survival

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SUMMARY

As a keystone species, honey bees (*Apis mellifera*) are pollinators that help sustain our food supply and native ecosystems. Unfortunately, habitat loss and widespread pesticide use are major drivers of pollinator decline. In the case of honey bees, rates of infection and colony collapse have been attributed to several interacting factors, including the loss of forage diversity and abundance. In this study, we aimed to investigate the effect of plant forage on bee health across apiaries located in multiple US cities. Hives were divided into healthy green zones, average health yellow zones, and unhealthy red zones. We hypothesized that honey bee colony survival would increase with the number of native plants foraged. Using plant DNA metabarcoding of honey samples, qualitative and quantitative analyses revealed hive health variation due to the population of plants foraged upon. Forage from green zones consisted predominantly of native plants and red zones consisted of primarily invasive plants. Furthermore, hives that were exposed to a natural catastrophic event demonstrated a high percentage of native plant forage post-disaster. Our study represents the first investigation of the significance of native and invasive plant forage to overwintering survival for honey bees as well as novel research examining the effect of natural catastrophic events on honey bee foraging. The availability of native and invasive plants plays a critical role in bee health, performance, and fitness, particularly in post-catastrophic event landscapes. By further understanding the unique dynamics between the type of plant forage and honey bee survival, we may be another step closer to unlocking the mysteries that may benefit the health of 200,000 other pollinator species.

INTRODUCTION

Honey bees (*Apis mellifera*) are crucial pollinators of 80% of flowering plants and more than 130 cultivated crops (1). In fact, bee pollination accounts for approximately 15 billion United States (US) dollars in added crop value (2). Additionally, bees also produce honey, pollen, royal jelly, beeswax, propolis, and venom for nutritional and medicinal uses for an additional 300 million US dollars annually (3). Due to their strong impact on pollination, honey bees are a keystone species within the ecosystem, often acting as bioindicators of the health of the environment (4). However, honey bees are facing more stressors than ever before and are declining at a rapid rate (5). Losses are attributed to Colony Collapse Disorder, which occurs when there is a sudden loss of a colony's worker bee population, yet the queen, brood, and a relatively abundant amount of honey and pollen reserves remain (5).

The main sources of nutrition for honey bees are floral resources including nectar (which is the primary source of carbohydrates) and pollen (the main source of protein) both of which are collected by worker bees (6). Nutritional requirements vary by role in the colony, as foragers (collect food resources) and nurse bees (process food and provisioning of larvae) require different nutrition, and overall foraging intensity is modulated at the colony level (7,8). Efficient colony maintenance and brood rearing require not only a sufficient quantity of pollen and associated nutrients, but also a diverse pollen diet, which is associated with notable benefits (9). Colonies dependent upon a single mono-floral crop, such as those often found in agricultural habitats, experience a brief glut of pollen (9). However, colonies may continue to struggle for sufficient nutrition at other times and are particularly susceptible to a failed crop or inclement weather (10, 11). A diverse diet of plants with flowering times spread throughout the season offers security against these risks, allowing increased temporal stability of nutrient availability (12). A diverse diet is also better able to meet the differential nutritional requirements of the different roles within the colony (13). In addition, a poly-floral diet can increase the immunocompetence of bees and has been indirectly associated with reduced disease and pesticide susceptibility of the colony (14, 15).

Native plants contribute to the diet diversity for honey bees. Native plants are especially important to the integrity of our ecosystem (16). A native plant species occurs naturally in a particular region, state, ecosystem, and habitat without direct or indirect human actions (16). Species native to North America are generally recognized as those occurring on the continent prior to European settlement. Native plants are key elements to sustaining rich and functionally diverse insect communities (17, 18). In contrast, non-native plants are plants that have been introduced to an area from their native range either purposefully or accidentally (16). Non-native plants can have adverse effects on ecosystem structure and processes by invading and out-competing native plants. Invasive non-native plants can dramatically alter the structure and dynamics of native plant communities and the functioning of ecosystems (19 - 21). Competitive interactions between invasive plant species and native plant species are one of the mechanisms underlying the impact of invasive plants in terrestrial ecosystems (19). Strong competition from invasive plants often leads to declines in the abundances, dominance, and, in certain cases, to localized extinctions of native plant

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species (19, 22, 23).

There is currently limited understanding surrounding the response of native plants and honey bees to the recovery of plant communities in natural post-catastrophic event landscapes. Because post-catastrophic events can open forest canopies and increase space and resources for understory flowering plants, there may be an effect on floral composition and honey bee foraging response (24). Previous studies have shown floral abundance and diversity tend to peak one to five years after a natural disaster event with prolonged flowering and increased nectar concentrations (24). A prolonged flowering season, often sustained by diverse and abundant flowers may be especially significant for honey bees. Poly-floral plants, which contribute to the forage of a hive, are generally characterized by the identification of pollen sources derived from hive pollen traps, isolates from honey, or via the physical tracking of foraging bees (25 - 27). DNA metabarcoding utilizes DNA extracted from honey to identify the floral composition of honey and has been shown to have benefits over other methods (28 – 30). While plant species identified from pollen loads give a direct measure of the plants visited by bees when collecting pollen, information from honey-extracted plant DNA can be used to describe plants visited for both pollen and nectar collection; although, some foraging is known to target pollen only and may therefore be missed when honey-based sampling is used (31 - 33). DNA barcoding utilizes a short section of DNA from a specific gene or several genes for species identification (28 - 30)**.** Several gene regions (e.g., *rbcL, trnL*) have low mutation levels and have been identified for use as metabarcodes in plants (33 - 36). A multi-gene region metabarcoding approach has also been recommended to increase the discriminatory power and broaden the range of species detection, as specific gene regions show biases in detection range and level of plant taxon identification (34 - 37).

In our study, DNA metabarcoding of honey samples of the surrounding landscape was used to determine the diet diversity and composition of the plants visited by honey bees in healthy green zones (≥ 80% overwintering survival), average health yellow zones (80% > x > 45.5% overwintering survival), and unhealthy red zones (≤ 45.5% overwintering survival). Overwintering is the process of bee survival during the cold season. The national overwintering average of 45.5%, as determined by the Bee Informed Partnership, was utilized as the delineating margin between the "green", "yellow", and "red" zones (38). This study also examined the plant communities and honey bee forage from natural post-disaster landscapes. We hypothesized that honey bee colony survival would increase with the number of native plants foraged. Using DNA metabarcoding analyses, our investigation specifically examined the forage diversity prior to and following Category 5 Hurricanes Irma and Maria in Humacao, Puerto Rico. Honey DNA showed native plants emerge first post-event. The aim of the data derived from honey DNA metabarcoding addresses the possibility of predicting honey bee overwintering success based on the type and proportion of plants (native, non-native, and invasive) foraged and also enabled an investigation into the effect of a natural post-catastrophic event on the native and invasive plant forage of honey bees.

RESULTS

Scientific beekeepers employed by The Best Bees Company installed and fully managed all beehives in this study. Hives were located at residential gardens, business rooftops, and institutional campuses. The Best Bees Company's proprietary national database, Bzzz, was utilized to collect overwintering data. The Bzzz database contains 3,536 *Apis mellifera* (European honey bee) hives installed between 2010 and 2022 in 21 greater metropolitan regions in the United States. Fifteen of the 21 metropolitan areas have accessible overwintering survival rates and honey DNA metabarcoding results (**Appendix A**). All beekeeping visits and data collection were conducted by over 200 experienced beekeepers using standardized methods for beekeeping practices as described by The Best Bees Company (38).

Plant Forage by Overwintering Survival Region: Qualitative Analysis

In an assessment of available data on the national Bzzz dataset, we calculated honey bee overwintering survival rates for 15 regions by averaging the last two years of overwintering percentages. Areas where bee health was significantly above average (≥ 80% overwintering survival) were labeled as "Green Zones", areas where bee health was moderately above average (80% > x > 45.5% overwintering survival) were labeled as "Yellow Zones", and areas below average (≤ 45.5% overwintering survival) were labeled "Red Zones" (**Table 1**). Each region was then sorted using the national overwintering average of 45.5% as the delineating margin between the three groups (37).

Using DNA metabarcoding analysis of the honey samples, a qualitative comparison of honey samples designated as green/yellow/red zones illustrates the percentages of native, non-native, and invasive plants from each zone (**Figure 1**). Green zones with healthy overwintering survival had the greatest percentage of native plants at 48.2%. Red zones with the lowest overwintering survival had the lowest percentage of native plants at 24.4% and in intermediate overwintering survival yellow zones, 28.4% of native plants were detected. In contrast, zones with the greatest percentage of invasive plants had the lowest percentage of overwintering survival. Red

City, State	Overwintering Survival Percentage	Zone Designation
Portland, OR	8%	Red
Seattle, WA	10%	Red
Washington, DC	25%	Red
Boston, MA	25%	Red
Chicago, IL	40%	Red
Oklahoma, OK	50%	Yellow
Pittsburgh, PA	64%	Yellow
New York NY	65%	Yellow
Philadelphia, PA	70%	Yellow
Dallas, TX	75%	Yellow
Denver, CO	85%	Green
San Francisco, CA	90%	Green
Los Angeles, CA	95%	Green
Ithaca, NY	95%	Green
Columbus, OH	100%	Green

Table 1: The Best Bees Company overwintering survival (%) and zone designation for *A. mellifera*. (N = 15). Table demonstrates the designation of "red, yellow, green" zones based on bee health as measured by overwintering survival rates for 15 US metro areas, encompassing a 90-mile distance from downtown centers. Regions with substantially above average bee health (≥ 80% overwintering survival) are designated "Green Zones", areas with moderately above average bee health (80% > x > 45.5% overwintering survival) are designated "Yellow Zones", and areas with below average bee health (≤ 45.5% overwintering survival) are designated as "Red Zones."

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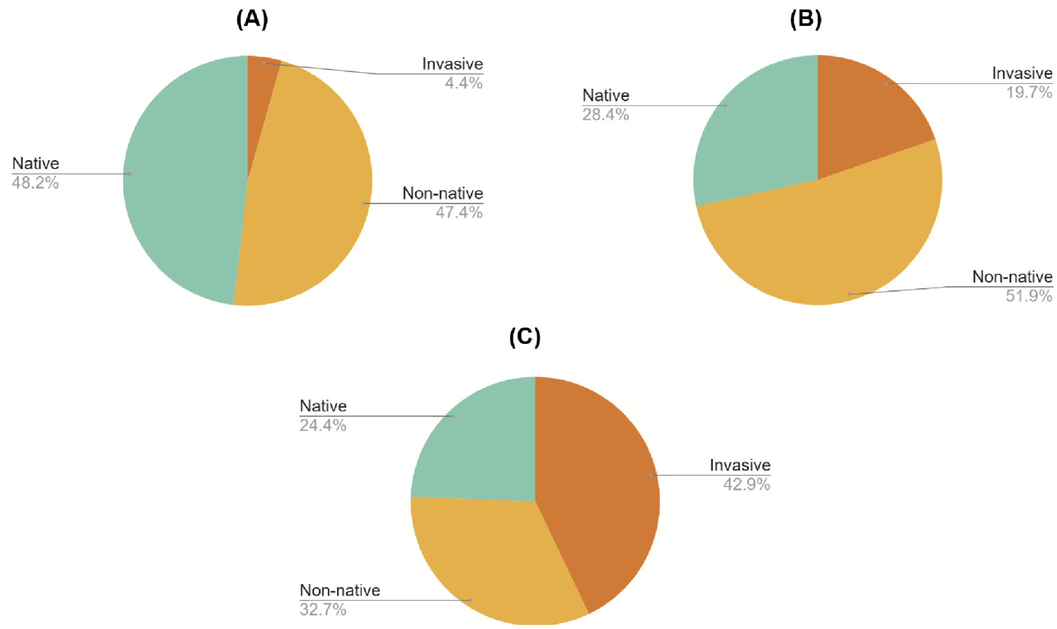

Figure 1: Composite plant percentages across green, yellow, and red zones demonstrates significant differences in the percentage of native and invasive plants foraged by *A. mellifera*. Pie charts depict healthy green zones had the highest percentage of native plants (1a), moderate yellow zones had the highest percentage of non-native plants (1b), and unhealthy red zones had the highest percentage of invasive plants (1c).

zones with the lowest overwintering survival had the highest percentage of invasive plants (42.9%), with 19.7% of invasive plants occurring in intermediate yellow zones. Green zones with healthy overwintering survival had the lowest percentage of invasive plants (4.4%).

Forage by Overwintering Survival Region: Quantitative Analysis

For the quantitative analysis, we compared the native, non-native, and invasive plants in each of the overwintering zones (green, yellow, red) using the Kruskal-Wallis, Dunn's (Mann-Whitney U), and Spearman Rho's Correlation Regression analyses. It is important to note that native plants may be invaded and out-competed by non-native plants; in particular, invasive non-native plants can dramatically alter native plant communities (18-22).

For invasive plants in each of the green, yellow, and red zones, a Kruskal-Wallis Test was performed to compare the number of invasive plants in each of the green, yellow, and red zones. A significant relationship was found between zone health and the forage of invasive plants (p-value = 0.003) (**Figure 2**). A more sensitive post-hoc Dunn's (Mann-Whitney U) test revealed healthy green zones and unhealthy red zones were also significant (p-value = 0.0006) (**Appendix B**). In order to determine the correlation between colony survival and the number of invasive plants foraged by honey bees, a Spearman Rho's Correlation Regression was performed and a correlation (p-value = 0.00008) between invasive plants and colony survival found indicating as the number of invasive plants increases, colony survival decreases across all zones (**Figure 3**).

A Kruskal-Wallis Test was performed comparing the number of non-native plants in each of the green, yellow, and red zones. For non-native plants, there was no difference (p-value = 0.4) across overwintering in all zones (**Figure 4a**).

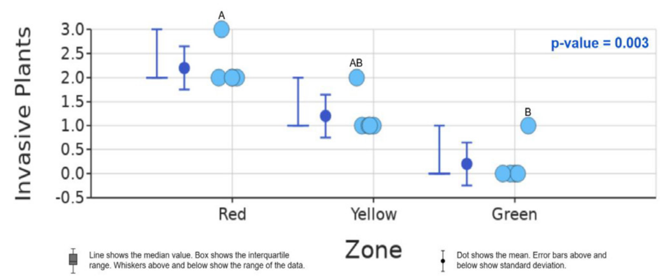

Figure 2: Invasive plant foraging is highest in unhealthy overwintering zones. Scatter plot with descriptive statistics reveals the number of invasive plants was statistically different across the red, yellow, and green zones (Kruskal-Wallis test, p-value = 0.003).

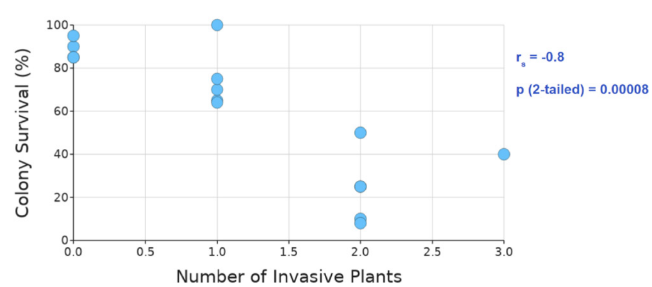

Figure 3: Inverse relationship between the number of invasive plants foraged and colony survival across all zones. Scatter plot demonstrates the number of invasive plants increases as colony survival decreases across all zones (Spearman Rho correlation regression, p-value = 0.00008).

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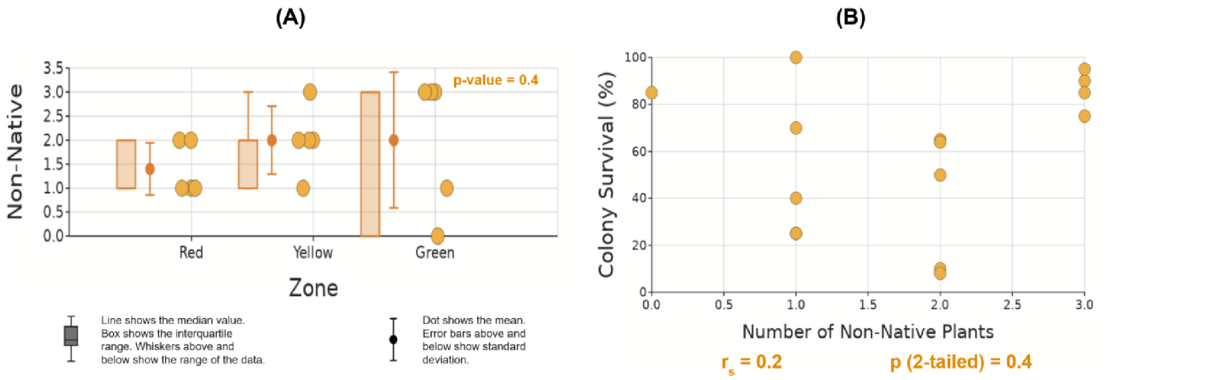

Figure 4: Across all zones, no relationship found between non-native plant forage and colony survival. 4a) Scatter plot with descriptive statistics depicts the Kruskal-Wallis test for non-native plants demonstrates no statistically significant difference in the number of non-native plants between zones. 4b) Scatter plot depicting the Spearman Rho correlation regression for non-native plants demonstrates no significant trend between the number of non-native plants and colony survival (%).

A more sensitive post-hoc Dunn's (Mann-Whitney U) Test (**Appendix C**) and Spearman Rho's Correlation Regression demonstrated no relationship (*p-value=0.3*) between non-native plants foraged and colony survival (**Figure 4b**).

For native plants in green, yellow, and red zones, a Kruskal-Wallis Test found no correlation (*p*-value = 0.08) with overwintering (**Figure 5**). However, the more sensitive post-hoc Dunn's (Mann-Whitney U) Test (*p-value = 0.03*) (**Appendix D**) and Spearman Rho's Correlation Regression for native plants revealed(*p*-value = 0.02) as the number of native plants increased, colony survival also increased across all zones (**Figure 6**).

Impact of catastrophic natural event on honey bee foraging

In an effort to determine the forage diversity and impact following a natural disaster event, DNA metabarcoding of honey samples were analyzed before and after a natural catastrophic event. DNA metabarcoding of honey samples taken in May 2017 from Humacao, Puerto Rico prior to Category 5 Hurricane Irma (September 5–7, 2017) and Category 5 Hurricane Maria (September 19–21, 2017) (**Appendix E**) were compared to honey samples taken in May 2018 (**Appendix F**) to examine differences in plants foraged (**Figure 7**). Prior to the 2017 Category 5 Hurricanes Irma and Maria, honey samples revealed a limited quantity of plant forage with 44.4% invasive plants, followed by 46.8% non-native plants, and 7.2% native plants. Following the 2017 catastrophic Category 5 Hurricanes of Irma and Maria, honey samples revealed a diverse quantity of plant foraged by honey bees with 72.4% native plants, followed by 23.0% non-native plants, and 3.5% invasive plants. No overwintering survival data was recorded on the Bzzz database for Humacao, Puerto Rico therefore quantitative analyses could not be completed.

DISCUSSION

As a keystone species, honey bees play a critical role in biodiversity, preserving ecosystem health, and advancing our understanding of the 200,000 other species of pollinators. Examining the significance of foraged plants and the effect of honey bee nutrition and survival serves as an impetus for the planting of native plants to support healthy ecosystems. Honey bees ensure the continued survival of not just the plants they pollinate but also of all the other organisms that rely on those plants for survival. Due to their significant economic and agricultural impact, investigations into forage diversity and abundance as a driver of honey bee decline is essential. Given their role as an indicator species, the implications of honey bees in our study may be used as a model to infer important concepts to the conservation of all pollinators.

Honey bees exhibit selectivity in their foraging of plant species due to the availability of resources and the needs of

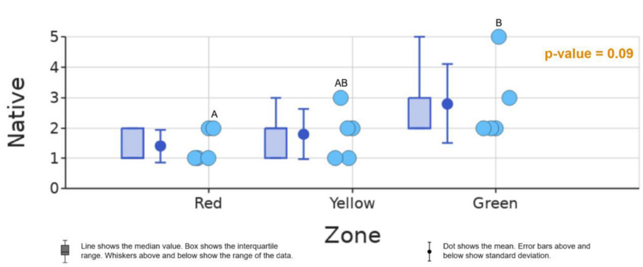

Figure 5: More sensitive quantitative test reveals native plant foraging is highest in green zones. Scatter plot with descriptive statistics reveals the Kruskal-Wallis test for native plants does not demonstrate a statistically significant difference in the number of native plants between zones.

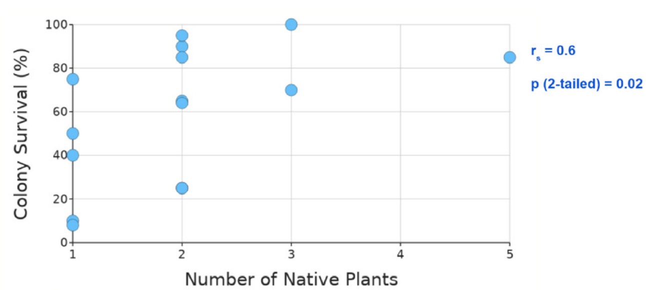

Figure 6: A positive correlation exists between the number of native plants foraged and colony survival (%). Scatter plot demonstrates the Spearman Rho correlation regression which demonstrates as the number of native plants increases, colony survival increases as well across all zones (*p*-value = 0.02).

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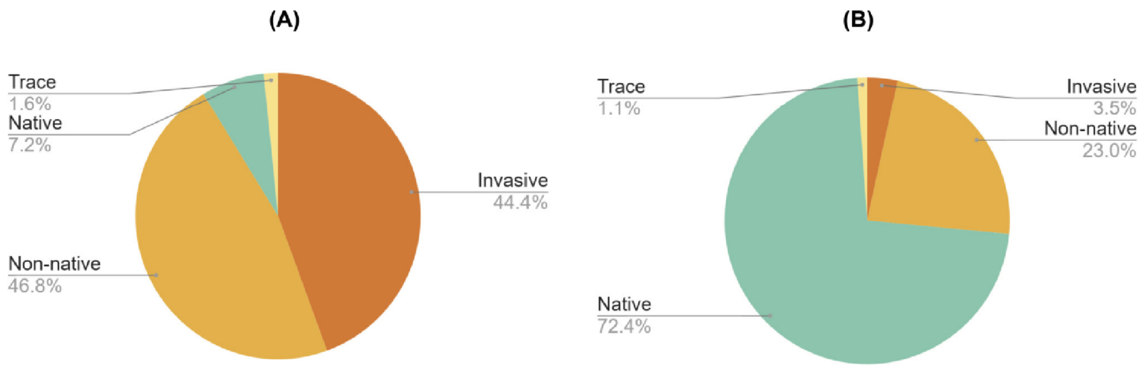

Figure 7: Composite plant percentages prior to and following 2017 hurricanes in Humacao, PR demonstrate significant differences in the percentage of native and invasive plants foraged by *A. mellifera*. Pie charts depict 7a) Plant percentages prior to the 2017 hurricanes, 44.4% of the honey bee forage consisted of invasive plants with only 7.2% native. 7b) Plant percentages following the hurricanes, 72.4% of the honey bee forage consisted of native plants and 3.5% invasive.

the colony. The designation of geographic regions into green, yellow, or red zones based on overwintering survival success found that native plant taxa richness is significantly correlated with overwintering survival. Hives with ≥ 80% overwintering survival (green zones) had the greatest percentage of forage derived from native plants with 48.2%. In contrast, hives with ≤ 45.5% overwintering survival (red zones) had the lowest percentage of forage from native plants with 24.4%. Furthermore, foraging derived from invasive plant taxa was inversely correlated with overwintering survival. Red zones with the greatest percentage of invasive plants at 42.9% had the lowest percentage of overwintering success. Green zones which had the lowest percentage of invasive plants at 4.4% had the highest percentage of overwintering success.

Plant DNA metabarcoding of honey samples demonstrated the relationship between hive health and the type of plants foraged upon by honey bees. For invasive plants, quantitative results revealed that colony survival and the number of foraged invasive plants were statistically significant across all zones, with the greatest disparity between healthy green zones and unhealthy red zones. As the percentage of invasive plants increased, colony survival decreased across all zones. For foraged native plants, quantitative results initially revealed no statistical significance between the zones; however, through the use of more sensitive analyses colony survival and the number of foraged native plants was found to be statistically significant across all zones, with the greatest disparity between healthy green zones and unhealthy red zones. As the percentage of native plants increased, colony survival increased across all zones.

Comparing honey sample foraging prior to and following a natural disaster revealed that in the aftermath of a natural catastrophic event, honey bee forage reverts back to native plants. The honey DNA metabarcoding qualitative results of hive forage prior to the 2017 Category 5 Hurricanes Irma and Maria, from Humacao, Puerto Rico, revealed forage consisted of 44.4% invasive plants, 46.8% non-native plants, and 7.2% native plants. However, following the hurricanes, foraging reverted back to native plants. Post-hurricane forage was comprised of 72.4% native, 23% non-native, and 3.5% invasives. In circumstances with limited growth resources, native plants use resources more efficiently than invasive plants, suggesting that native plants have a higher ability to compete against invasives in a resource-poor habitat. Currently, no other research has examined the effect of native or invasive plant forage on overwintering survival for honey bees as well as the effect of natural catastrophic events on honey bee foraging.

Our novel study demonstrated the use of DNA metabarcoding can identify honey bee forage after a natural catastrophic event. In an investigation of the forage prior to and after Category 5 Hurricanes Irma and Maria, the post-hurricane forage consisted primarily of native plants. This study represents the first investigation of the use of honey DNA metabarcoding to realize the significance of native and invasive plants forage on overwintering survival for honey bees. DNA metabarcoding of honey samples demonstrated qualitative and quantitative statistically significant outcomes in the composition of plants visited by honey bees. Our analysis revealed that the honey bee diet is focused on a variety of plants. The type of plants foraged, native or invasive in origin, affected the success of honey bee overwintering and survival. Using both qualitative and quantitative analyses, this study suggests the greater the dependence on native plants, the higher the percentage of overwintering survival. Likewise, the greater the dependence on invasive plants, the lower the percentage of overwintering survival.

A closer examination of our investigation did include limitations. While The Best Bees Company's beekeeping practices are standardized, there is some degree of variation between regions and beekeepers that could perhaps play an unanticipated role in the bee health outcomes. Furthermore, the novel "green, yellow, and red" zone categorization concept enabled the simplification of the definition of bee health. While overwintering survival was utilized as the primary measure of bee health, summer beehive deaths were not recorded. The consideration of other survival measures should also be considered. Successful overwintering survival is the result of a highly complex interplay between ecology, evolution, and behavior; although, the complete investigation of these interactions was beyond the scope of this investigation.

As a pioneering study, opportunities abound for additional investigation. The assessment of native and invasive plants and overwintering survival should be expanded into other cities. Because this research was limited to the US only, an examination of this interplay in other countries and continents may reveal additional relationships due to differing native, non-native, and invasive plant species. Because previous investigations have revealed the dietary preferences of honey bees for non-native plants, explorations into the specific nutritional benefits of native, non-native, and invasive plants may expose other significant correlates, perhaps related to nutritional requirements and lifecycle, to unveil future targets for advanced study (39, 40). Currently, the Bzzz database summarizes the foraging diversity in designated regions; however, the dataset was not hive-specific, thus future studies may be explored at the colony level for patterns and relationships. The use of honey DNA metabarcoding may also be studied to include propolis DNA to account for foraging opportunities derived from plant resins. Further investigations of plant foraging prior to and following other natural catastrophic disasters arising from earthquakes, wildfires, and floods, as well ramifications from the COVID-19 lockdown, may also be studied. Although overwintering survival data prior to and following the hurricanes in Humacao, Puerto Rico was not available for this investigation, future explorations examining other natural catastrophic events should include overwintering survival data to allow for subsequent quantitative analyses. Perhaps natural disasters may serve as a "reset" across life species to restore natural states and improve ecosystem health. Future research may extend this investigation to explore if pollinator populations may be healthiest after natural disasters due to the abundance of native plants which coincides with high-quality nutrition to empower bees to withstand disease and better metabolize pesticides. With advancing technological approaches in machine learning, artificial intelligence, data analytics, and in-hive advances, "Smart Hives," will likely establish honey bees as the gold standard model system for increased precision in data collection.

MATERIALS AND METHODS

All honey sampling took place during 2019–2023. Samples from each region were collected using sterile, plastic test tubes to scoop honey directly from the comb in the hive. Honey harvested from shared equipment was not utilized due to the potential for contamination during the extraction process. For DNA metabarcoding analysis, pollen within the honey was analyzed using genomic sequencing to identify the foraged plant species. All genomic DNA was extracted following manufacturer's guidelines provided by the MoBio PowerSoil htp-96 well Isolation Kit (Carlsbard, CA) and all samples were analyzed using chloroplastic trnL markers (41). Based on the identified taxa results from DNA metabarcoding, all identified foraged species were sorted into native, non-native, or invasive plants through the USDA PLANTS Database (42). The national overwintering average of 45.5% from the Bee Informed Partnership was utilized as the delineating margin between designated zones (38).

For the qualitative analyses, areas where bee health was substantially above average (≥ 80% overwintering survival) were labeled as "Green Zones", areas where bee health was moderately above average (80% > x > 45.5% overwintering survival) were labeled as "Yellow Zones", and areas below average (≤ 45.5% overwintering survival) were labeled "Red Zones".

For the quantitative analyses, using SSPS software, both a Shapiro-Wilk Test and a Kolmogorov-Smirnov Test were run to test for non-parametric data. Due to the non-parametric data, a Kruskal-Wallis Test followed by post-hoc Dunn's Test (Mann-Whitney U Test) was performed. Additionally, a Spearman's Rho Correlation Regression was performed in place of a Linear Regression.

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APPENDIX

Common Name	Scientific Name	Designation	Percentage in Designation	Percentage in Total
Clover	*Trifolium*	Non-native	25.94%	11.66%
Crape Myrtles	*Lagerstoemia*	Non-native	17.97%	8.08%
Magnolia	*Magnolia*	Native	18.92%	6.06%
White Sweet Clover	*Melilotus albus*	Invasive	25.60%	5.9%
Rose	*Rosa*	Non-native	13.01%	5.85%
Legume	*Fabaceae*	Native	13.86%	4.44%
Pine	*Pinus*	Native	13.07%	4.18%
Tree of Heaven	*Ailanthus altissima*	Invasive	10.37%	2.39%
Yellow Sweet Clover	*Melitous officianalis*	Invasive	8.23%	1.90%

Appendix A. Most common native, non-native, and invasive plants derived from DNA metabarcoding of honey samples of honey bee forage reflecting the diverse and poly-floral diet of *A. mellifera*. Table reveals the type of forage, native, non-native, invasive plants, derived from honey DNA metabarcoding of honey bee colonies identified from 15 US cities.

Pair	Mean Rank Difference	Z	SE	Critical Value	p-value
Red-Yellow	4.6	1.7	2.7	6.5	0.09
Red-Green	9.2	3.4	2.7	6.5	**0.0006**
Yellow-Green	4.6	1.7	2.7	6.5	0.09

Appendix B. Post-hoc Dunn's analysis (Mann-Whitney U Test) of invasive plant foraging. The analysis reveals a significant difference in invasive plants between the red and green zones specifically (p-value = 0.0006).

Pair	Mean Rank Difference	Z	SE	Critical Value	p-value
Red-Yellow	-2.9	1.1	2.7	6.5	0.3
Red-Green	-3.1	1.1	2.7	6.5	0.2
Yellow-Green	-0.2	0.07	2.7	6.5	0.9

Appendix C. Post-hoc Dunn's analysis (Mann-Whitney U Test) of non-native plant foraging. The analysis demonstrates no significant difference in the number of non-native plants between all zones**.**

Pair	Mean Rank Difference	Z	SE	Critical Value	p-value
Red-Yellow	-2.1	0.8	2.6	6.3	0.4
Red-Green	-5.7	2.1	2.6	6.3	**0.03**
Yellow-Green	-3.6	1.4	2.6	6.3	0.2

Appendix D. Post-hoc Dunn's analysis (Mann-Whitney U Test) of native plant foraging. The analysis demonstrates a significant difference in the number of native plants between red and green zones specifically (p-value = 0.03).

Common name	Designation	Percentage of Forage
Clover	Non-native	46.80%
Trefoil	Invasive	44.42%
Bignonias	Native	7.16%
Trace *		1.62%

*quantities too small to be identified

Appendix E. Prior to (before) 2017 category 5 hurricanes Irma and Maria exemplifies the differences in the quantity and type of *A. mellifera* plant forage before the hurricanes. Table demonstrates the type and quantity of forage derived from DNA metabarcoding of honey prior to 2017 hurricanes in Humacao, Puerto Rico.

Common name	Designation	Percentage of Forage
Melothria	Native	33.94%
Spurges	Native	20.53%
Bitter Melon	Non-native	11.78%
Blackbeads	Non-native	6.64%
Cucurbitales	Native	5.52%
Asparagus	Non-native	4.61%
Snakeroots	Native	4.55%
Arecales	Native	4.02%
Tropical Almond	Invasive	3.46%
Melonleaf	Native	2.30%
Nelsonia	Native	1.54%
Trace *		1.11%

*quantities too small to be identified

Appendix F. Following (after) 2017 category 5 hurricanes Irma and Maria exemplifies the differences in the quantity and type of *A. mellifera* plant forage after the hurricanes. Table depicts the type and quantity of forage derived from DNA metabarcoding of honey after 2017 hurricanes in Humacao, Puerto Rico.