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Quantitative and Qualitative Nutritional Analysis of GVSU Managed Honey Bee Colonies

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Abstract

Managed honeybee colonies are in significant decline worldwide. The interaction between poor nutrition, pests and diseases, and pesticide use are most cited as potential culprits for the precarious state of the beekeeping industry. By evaluating food coming into the hive, conclusions can be drawn about the quality of hive location and forage availability. Pollen from an apiary with historically low honey production and poor colony health was compared to pollen from an apiary with high honey production and good colony health. Pollen was collected weekly in a 24-hour period, hive weight was monitored, and colonies were assessed for overall growth and health. There was no significant difference in pollen diversity or crude protein content between the study sites; however, there was a significant difference in the quantity of pollen collected. Colony production was also comparable. A mobile application was developed as a tool for beekeepers to replicate this research using similar protocol and to participate in pollen data gathering as citizen scientists. This will allow for the collection of broad geographic data on a larger scale.

Introduction

In recent years, honeybee colonies have suffered unusual losses. Many interrelated factors are likely the cause (Neumann and Carreck, 2010). Among the main stressors are pests and pathogens (such as *Varroa destructor*), pesticides, loss of forage, and beekeeping practices (Neumann and Carreck, 2010). When these factors are combined, colonies often experience lower rates of overwintering success and higher susceptibility to infection and other stress year round (Neumann and Carreck, 2010).

While many studies focus on pesticide uses as well as pests and diseases and their effects on honey bees, studies on nutritional deficiencies resulting from growing monoculture practices are less prevalent in the literature. Modern agricultural landscapes, for example, can be especially resource poor for pollinators, as they often contain little diversity in flowering plants (Blaauw and Isaacs 2015; Brodschneider and Crailsheim, 2010). A heterogeneous landscape, where bees have been found to survive best, usually indicates more diverse forage and better nesting sites (Winfree et al., 2007).

Colony health is heavily dependent on regular access to quality and quantity of nectar, which supplies energy in the form of carbohydrates, and pollen, which supplies protein (Wratten et al., 2012). Colonies require income of sufficient protein in order for proper brood development (vanEngelsdorp et al., 2009; Haydak, 1970). Protein is essential in the

maturation of flight muscles, reaching maximum thorax mass, and the development of hypopharyngeal glands and ovaries (Brodschneider and Crailsheim, 2010). Nurse bees that rear brood without sufficient protein intake utilize their own body content to process larval food, which results in a drop in weight and body nitrogen (Haydak, 1970). Nurse bee health is important for the maintenance of colony strength. When older bees are forced to rear brood, the quality of the larval food they produce decreases, which negatively affects the development of emerging bees, the longevity of these bees, and the weight and nitrogen content of reared queens (Haydak, 1970).

Though it has been found that bees can adjust to pollen dearth for up to 60 days, (Haydak, 1970) finding diverse, high quality pollen can be difficult throughout an entire blooming season. Diets of mixed pollens are usually considered superior to single pollen diets, with the exception of uniform diets of sweet clover or mustard (Brodschneider and Crailsheim, 2010). In fact, Alaux et al. (2010) found that a diet diverse in pollen was the main factor in colony immunocompetency. Specific amino acids must be present in the pollen gathered in order to assure proper development (Haydak, 1970; Brodschneider and Crailsheim, 2010), and not all plant species provide a complete suite of essential amino acids, the building blocks of protein. Necessary nutrients that are not available in one type of pollen can be provided by another (Brodschneider and Crailsheim, 2010), thus diverse pollen sources likely indicate a more adequate diet.

This study compared pollen quantity and quality in color diversity and crude protein content in two different locations in order to evaluate forage availability throughout the season. This was superposed with assessments of colony growth. We predicted that the apiary located amongst intensive conventional agriculture would have lower quality of food, thus lower health, than the apiary located in a natural unmanaged prairie. This study also aided the development of a protocol for the collection of broad geographic data and the future development of a mobile application.

Materials and Methods

Study site

This study was conducted between the months of May and September 2016. Two research apiaries were used during the course of this study. One was located in Allendale, Michigan at Grand Valley State University's Sustainable Agriculture project. The other was located in Holland, Michigan at the GVSU Meijer Campus. The 'Allendale' apiary sits on a two-acre sustainable farm surrounded by intensive conventional agriculture of corn and soybean, while the 'Holland' apiary is located on a fifteen-acre unmanaged semi-natural prairie. These sites sit over 30 miles apart, ensuring that honey bee foraging location does not overlap.

Hive Inspections

For this study, hive inspections were performed bi-weekly. Brood pattern (quality on a scale from 1-5), number of frames of bees, queen status were recorded and annotated with any other pertinent information, including disease load, management practices, and treatments. Hive weight was recorded using SolutionBee hive scales, data uploaded to a mobile device in the field

and later to Bee Informed Partnership servers (http://hivescales.beeinformed.org server).

Pollen Collection

Pollen was collected using Sundance Pollen Traps. The traps were opened for 24-hour periods on a weekly basis to collect samples. We selected warm, sunny days for collection to insure optimal honeybee foraging.

Pollen Analysis

For each pollen sample collected, a wet weight and dry weight were recorded. Pollen samples were dried in a drying oven at 90°C for an hour. A subsample of 100 pollen pellets was then randomly selected from each total weekly sample, which was further sorted into pellet color categories. The number and weight of pellets in each color category were recorded. These individual color groups were massed and then scanned to keep record of color assortment. Pollen was placed on the scanner alongside a photography color balance card. The card acted as a reference to assure a standardized color spectrum between scans.

Microscopy was used to verify the accuracy of pollen diversity based on color count. Slides were made using a wet mount technique. To prepare the pollen for mounting, pellet color groups were vortexed with water for 30 seconds to form a homogenous mixture. Fifteen microliters of diluted safranin dye was then placed on a microscope slide, followed by the application of a small amount of pollen-water mixture using a toothpick. When analyzing slides, we noted the number of different pollen species observed. We did not identify pollen plant species, but simply determined the total diversity count of each 100-pellet subsample. These data were recorded as plus or minus the number of different species of observed pollen grains that were originally estimated by the color count method.

Pollen samples were also sent to Midwest Laboratories for protein analysis using the kjeldahl method. One sample from each research apiary was sent on a biweekly basis from May to August.

Diversity Index (DI)

A 'Diversity Index' (DI) was calculated for each sample. Considering that while the different number of colors could translate to an expression of diversity, the proportion of pellet in each color would be a more accurate representation of diversity overall. The (DI) for pollen grains was defined in the fashion similar to center of mass, or GPA. The DI is the mass-weighted average pollen type in a sample, with pollen types arbitrarily indexed by integers n = 0, 1, 2, ..., N-1 (N is the total number of pollen colors) in order of decreasing abundance. $DI = 2/(N-1) * (CI\_1 * m\_1 + CI\_2 * m\_2 + CI\_3 * m\_3 + ...) / (m\_1 + m\_2 + m\_3 + ...)$, in which N is the total number of pollen colors, CI_i is an integer starting at zero that arbitrarily labels each pollen color (sorted in order of decreasing abundance), and m_i is the mass of each pollen color. The factor 2/(N-1) normalizes the DI so that it tends toward 0 when only one pollen color is present in a sample and tends toward 1 when a sample of pollen contains equally abundant pollen colors. This DI can then be expressed in a diversity percentage value.

Statistical Analysis

All statistical figures were rendered using the Wilcoxon signed rank test. The Wilcoxon signed rank test was used because of small sample size and assumed symmetry. Between the two study sites we compared: 1) wet masses of weekly total pollen samples, 2) the number of colors of pollen pellets in a 100 pellet subsample, 3) the diversity index and 4) the crude protein content of pollen samples taken biweekly. A 95% confidence interval was used.

Results

We found no significant difference between locations in pollen diversity by color (Fig 1; Wilcoxon signed rank test: P-value > 0.05). When pollen samples were sorted by color, we found a range from one color to six colors throughout the entire blooming season. No pollen was collected in Allendale during the weeks of July 24th and 31st. During that time, hive inspection revealed a queenless hive status. After switching to a new hive for pollen collection on the week of July 22nd, we found that the bees still gathered from diverse plants species in August. The number of colors in each weekly sample tended to decrease in September in Allendale.

We also found no significant difference between the two sites in the proportion of pollen species in each sample based on the diversity index (Table 1; Wilcoxon signed rank test: P-value > 0.05). The diversity index ranged from 0-85% in Allendale and 8-77% in Holland (Table 1). On 8/7/16, the diversity index could not be calculated because the sample of pollen was too small to sort. The index could not be calculated during the week of 8/21/16 in Holland due to a lost sample.

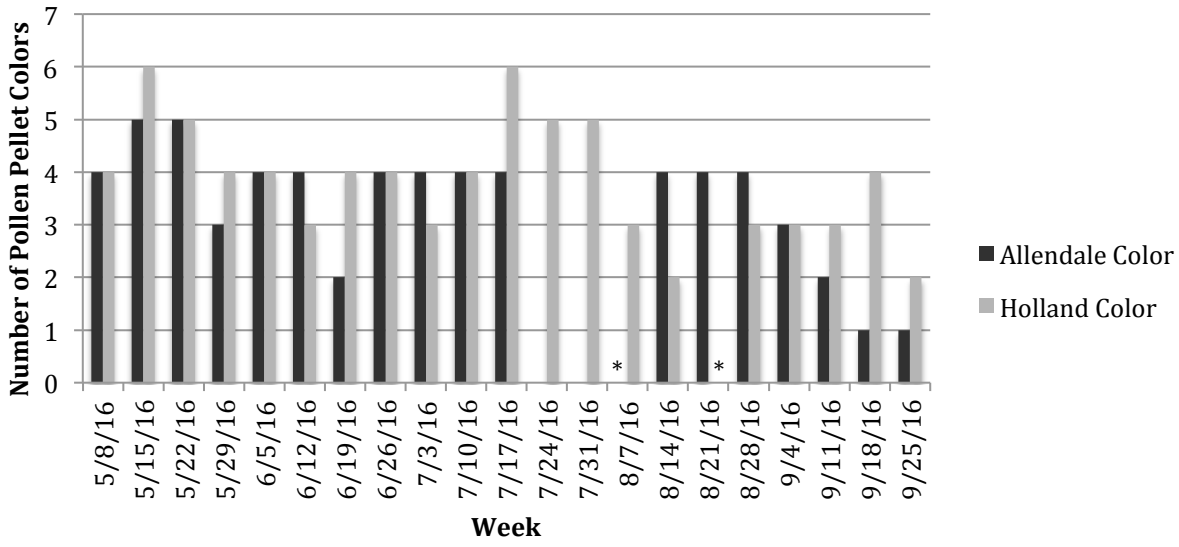

Figure 1. Number of colors of pollen pellets counted in a 100-pellet subsample gathered weekly from Holland and Allendale colonies (P-value=0.2633). The Wilcoxon signed rank test was used because of small sample size and assumed symmetry. Asterisks (*) denote either missing samples or samples too small to sort, while dates with no bars or asterisks are true zeros.

Table 1. Diversity Index (%) indicating the relative quality of the pollen sample by proportion of mass of each pellet color group (p-value=0.3165). The diversity index was calculated for both Holland and Allendale locations each week.

Weeks	Allendale Diversity Index (%)	Holland Diversity Index (%)
5/8/16	6%	23%
5/15/16	61%	49%
5/22/16	85%	49%
5/29/16	39%	8%
6/5/16	37%	62%
6/12/16	42%	44%
6/19/16	14%	53%
6/26/16	76%	26%
7/3/16	36%	31%
7/10/16	35%	37%
7/17/16	44%	50%
7/24/16	0%	28%
7/31/16	0%	40%
8/7/16	N/A	55%
8/14/16	33%	50%
8/21/16	49%	N/A
8/28/16	39%	21%
9/4/16	72%	20%
9/11/16	4%	22%
9/18/16	0%	42%
9/25/16	0%	77%

Table 2. Biweekly wet crude protein analysis of pollen selected randomly from the total samples from Holland and Allendale colonies (P-value=0.2246). The Wilcoxon signed rank test was used because of small sample size and assumed symmetry. These samples were collected from May through July only.

Weeks	Allendale Wet Crude Protein (%)	Holland Wet Crude Protein (%)
5/8/16	31.5	30.1
5/22/16	29.1	28.7
6/5/16	20	20.8
6/19/16	21.6	19
7/3/16	24.5	20.7
7/17/16	23.3	20.5
7/31/16	N/A	19.6

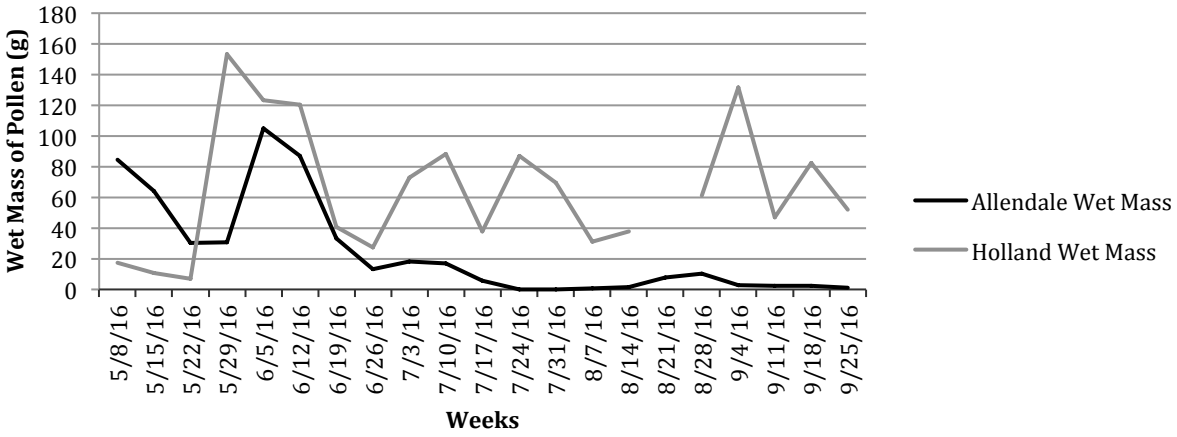

Figure 2. Wet mass of weekly total pollen samples collected from Holland and Allendale colonies (P-value=0.0005). The Wilcoxon signed rank test was used because of small sample size and assumed symmetry.

Crude protein content was also not significantly different between Holland and Allendale (Table 2; Wilcoxon signed rank test: P-value > 0.05), but seemingly decreased as the season progressed. The greatest difference in crude protein content between the two locations was 3.8%, which occurred during the week of July 3rd.

On the other hand, there was a significant difference between the Allendale and Holland colonies in the total weekly pollen sample wet mass (Fig 2; Wilcoxon signed rank test: P-value < 0.05). Overall, the Holland hive gathered more pollen than the Allendale hives. Again, we were unable to mass the Holland pollen sample for the week of 8/21/16 because the sample was lost.

Hive scale data indicated a steady increase in hive weight throughout the season in both locations (Fig 3 a., b.). In Allendale, pollen and scale data were collected from one hive until 07/22/16, and then another hive from 07/22/16 until the end of September. The sudden dips in weight in early August occurred because honey supers were taken off for extraction (Figure 3b).

Discussion

We hypothesized that the quantity and quality of nutrition in the Allendale apiary would be lower than that of the Holland apiary due to the greater agricultural landscape surrounding the Allendale hives. This hypothesis was not supported, as the results show that the quality of pollen was comparable between the two sites. The major focus of this study was to assess the diversity of plants available to our colonies on a weekly basis. We found no significant difference between the two sites in pollen diversity by color, nor were there any concerning gaps in diversity during any given period in the blooming season. Though we did not identify the species of plants from which the colonies were collecting pollen, a diversity count still provided information about nutrition.

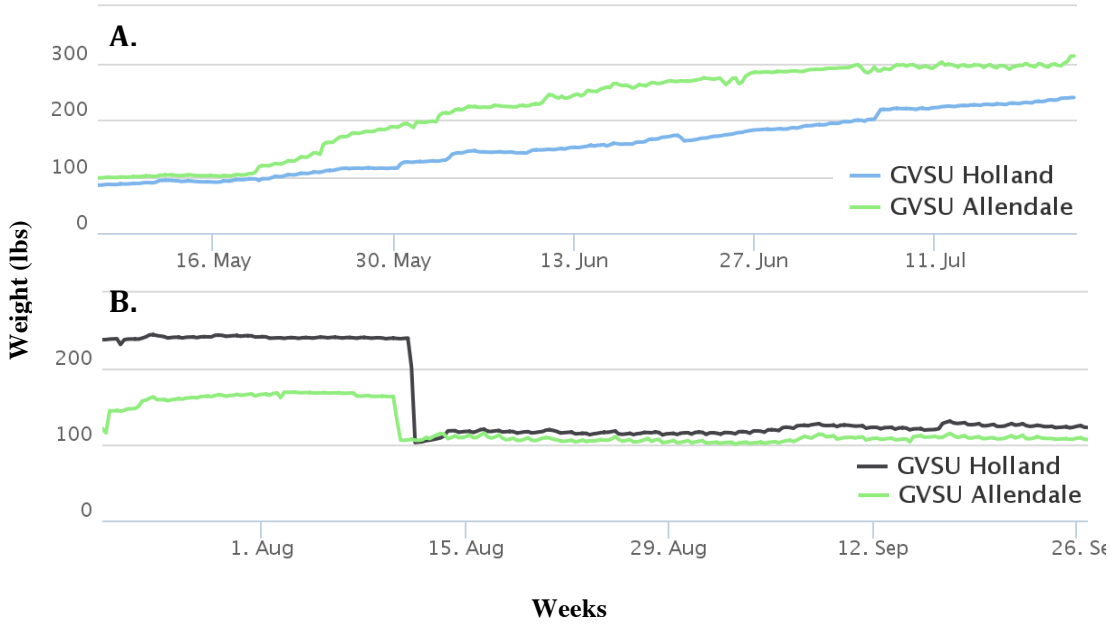

Figure 3. Weight of research hives in Allendale and Holland, Michigan from (A.) May 8th, 2016 to July 22nd, 2016 and from (B.) July 22nd, 2016 to September 30th, 2016. Figures taken from http://hivescales.beeinformed.org ; highcharts.com

Mixed pollen diets are generally considered superior to single pollen diets (Alaux et al., 2010; Brodschneider and Crailsheim, 2010; Haydak, 1970), as a more complex diet is more likely to provide the proper suite of amino acids to support colony health (Brodschneider and Crailsheim, 2010). Our results suggest that colonies at both sites had access to mixed pollen varieties throughout the majority of the Michigan blooming season.

Likewise, the diversity index showed no significant difference between locations. The diversity index was important to more precisely reflect the overall balance of our colonies' diets. It further distinguished between overall species diversity and the proportion of each species from which they were collecting. The index ranged from 0-85% in Allendale and 8-77% in Holland, indicating a wide range of diversity throughout the season. Overall, it appears that lack of pollen diversity in Allendale was not the factor causing poorer colony performance as originally predicted.

Not only does the diversity of pollen protein greatly influence colony health, the amount of pollen protein is also important. To estimate this, we tested for crude protein content, which also showed no significant difference between the two locations (although it has to be stressed that we tested a small sample size). Our crude protein content ranges from 19-31.5%, which aligns with the claim from Kleinschmidt and Kondos (1976) that pollen with crude protein content higher than 20% satisfies the nutritional requirements of a honeybee colony. This research is dated, and further studies that compare colony health and pollen crude protein content would help clarify our results. Pasquale et al. (2013) found that crude protein is not the sole indicator of nurse bee development, but lipid content also plays a large role. Perhaps

determining lipid content in our pollen samples along with other nutritional factors would further reveal details about pollen quality.

We also compared the quantity of pollen collected. Even if a pollen diet is diverse, too little pollen will not fully support a growing colony. Interestingly, we found that the Holland hive collected significantly more pollen than the Allendale hives. Though it seems that this would mean floral resources were more abundant in Holland, this is not necessarily the case. Our Holland hive did not adjust well to the pollen trap unless we kept it open 24/7, whereas our Allendale hive cooperated with the opening and closing of the trap. Because of this, we removed larger amounts of incoming pollen from the Holland hive, which may have caused them to recruit a higher proportion of pollen foragers to make up for their losses (Pernal and Currie, 2001). The difference in treatment of the pollen trap likely skewed the results for pollen quantity.

Studies vary widely in adequate pollen intake values, and have not been updated in recent years. According to Wille et al. (1985), honey bee colonies collect 10-26 kg of pollen annually, while Kleinschmidt and Kondos (1976) identify a 55 kg annual requirement for strong colonies in Australia. On average, a worker bee consumes 3.4-4.3 mg of pollen daily (Crailsheim, 1986). Another study determined that 125-187.5 mg of pollen is required to rear one larva (Hrassnigg and Crailsheim, 2005). We did not assess the required pollen per bee in this study, but this variability in literature values shows that much research is still necessary on the subject of pollen intake. We also do not have the ability to accurately estimate the total amount of pollen that each of our hives collected throughout the blooming season, because one 24 hour sample per week may not be completely representative of every day. Foraging behavior changes with the weather patterns throughout a week, thus affecting the amount of pollen being collected on a daily basis. Even so, the data collected from our scales support that our hives had enough pollen stores (as well as nectar stores) for continuous growth throughout the season. Regular hive inspections also confirmed that stores were adequate. Much smaller amounts of pollen are stored in colonies than honey, and periods of inadequate forage quickly deplete pollen stores (Schmickl and Crailsheim, 2001, 2002; Pernal and Currie, 2001); therefore, a dip in colony productivity would be apparent in tandem with a lack of pollen resources.

Though there was a dip in the amount of pollen gathered in Allendale in early August, this was likely because the hive became queenless in late July and not a result of a lack of forage. The absence of the queen causes a break in the brood cycle leading to an absence of uncapped brood and most likely the absence of the pheromone stimulus for pollen collection (Al-Tikrity et al., 1972) During the difficulty experienced in recuperating the Allendale research hive, we decided to begin collecting from a new hive. The increase in pollen quantity gathered after switching the trap to a neighboring colony shows that pollen resources were still abundant in Allendale, despite the decrease in pollen gathering of the queenless research hive.

Unlike we predicted, pollen quality was statistically the same in Holland and Allendale and pollen quantity was adequate at both sites. This could be attributed to the fact that Grand Valley State University's Sustainable Agriculture Project (SAP) in Allendale greatly

improved nearby forage by planting a field of native and non-native annual and perennial wildflowers. The literature supports that planting native perennial hedgerows like the ones established at the SAP could help provide more resources for pollinators (Isaacs et al., 2009; Decourtye et al., 2010). As well, Kremen et al. (2007) cites that a predominantly agricultural landscape can be beneficial for pollinators if it is heterogeneous, providing complex habitat and forage. This demonstrates that it is possible for the predominantly agricultural landscape in Allendale to be as resource rich as the unmanaged prairie in Holland.

One flaw in our experimental design is the use of only one pollen trap per apiary. This resulted in a small sample size and limited the possibility to make comparisons between hives in the same apiary. Previous studies show that neighboring colonies may forage from very different crop varieties (Synge, 1947), which could be due to competition and differences in land partitioning. In order to obtain a more accurate reflection of pollen diversity and quantity, at least two colonies should be sampled for pollen in each apiary. This helps to account for behavioral variability in the bees, as well as variability in the landscape, that may impact results.

Our original hypothesis stemmed from the observations that our Allendale hives historically did not perform as well as Holland hives in late summer months. If nutritional quality was not the cause of this observed difference as our results suggest, then it seems that some other environmental pressure(s) may be the cause. One such factor could be the presence of a high *Varroa destructor* population in Allendale. It is also possible that pollen and nectar gathered in an area prevalent with conventional agriculture could contain more pesticides (Chen and Mullin, 2013), which could have sublethal effects on colonies (Stoner and Eitzer, 2013; Li et al., 2015). The pesticide content of our pollen is another possible future experimentation. Overall, conducting more repetitions of this nutritional analysis and testing for more variables would create a clearer picture of what is affecting colony health negatively in Allendale and promoting it in Holland.

Conclusion

Though nutrition does not appear to be the factor responsible for decreasing colony health of the GVSU managed honey bee colonies in Allendale, nutritional analysis added to the understanding of how our colonies function, the composition of their surrounding landscape, and the forage available throughout the local blooming season. Honey bee nutrition is not widely reported in the literature, and those that are, are dated. Nutritional information is also missing from current national honey bee surveys conducted in the U.S. The protocol for this study will be used to create a mobile application that will allow beekeepers to replicate our experiment and participate in pollen data gathering as citizen scientists. The mobile application, called PollenCheck, is currently in development and will help gather information about pollen diversity and quantity on a broader geographic scale.

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