Impact of Hurricane Maria on mold levels in the homes of Piñones, Puerto Rico

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Abstract

Hurricane Maria struck Puerto Rico on September 20, 2017, severely impacting the island. In order to quantify the impact of the hurricane on the indoor air quality, we evaluated the fungal levels in households ($n = 20$) of the Piñones community for the period of 2018 and 2019. For each dust sample collected, the 36 Environmental Relative Moldiness Index (ERMI) molds were quantified using qPCR assays, and then Shannon Diversity Index (SDI) values for the fungal populations were calculated. Homes were in five separate regions, regarding their proximity in the studied area. We found that for regions with reported least water damage, the SDI values were similar for both sampled years, but for regions that reported mid-to-high level of damage region, the SDI values were significantly higher. Households that reported remediation actions between the two sampled years showed similar values for the second year as those that did not report any major impact. Our preliminary data provides insights into the significant impacts of hurricanes into indoor fungal environment.

Keywords Puerto Rico · Mold · Hurricane · Housing · Construction materials

Introduction

On the morning of Wednesday, September 20, 2017, Hurricane Maria struck Puerto Rico with a category 4 wind force and heavy rains. Much of the island was without power for months. The hurricane had devastating effects on the environment and people. These environmental impacts included destruction of the island's vegetation (Scholl et al. 2021) and housing infrastructure (Ma and Smith 2020). Renters and low-income families were found to be most vulnerable to Maria's impact (Ma and Smith 2020). With climate change, hurricanes are expected to increase in number and intensity with impacts on the environment and diseases like asthma (D'Amato et al. 2020; El-Sayed and Kamel 2020).

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Even before the hurricane, Puerto Rico had high rates of asthma, approximately 16.5% compared to 8.4% for mainly the United States (US) (US CDC 2012). In 2000, 30.6% of interviewed subjects reported having one to three visits to emergency departments as a result of asthma in the previous year (Pérez-Perdomo et al. 2003). In some areas of Puerto Rico, 46% of elementary children had asthma (Loyo-Berríos et al. 2006).

A study of healthcare utilization in Puerto Rico in 2013 demonstrated that increases in mold and PM_{10} were associated with increases in asthma claims (Lewis et al. 2020). In 2014–2016, an estimated 53% of adults with asthma and 29% of children with asthma in Puerto Rico had uncontrolled asthma (Cowan et al. 2020). Adults and children with uncontrolled asthma were significantly more likely to have observed mold in their homes than were those with controlled asthma. Blatter et al. (2014) suggested that indoor fungal exposure leads to an increased degree of atopy and visits to the emergency departments or urgent care facilities for asthma in Puerto Rican children.

Exposures to damp-indoor, moldy environments are linked to asthma based on many scientific reviews (IOM 2004; WHO 2009; Quansah et al. 2012; Sharpe et al. 2015). Therefore, it is important to have quantitative methods to describe mold contamination levels in homes. The US

Environmental Protection Agency in conjunction with the Department of Housing and Urban Development created the Environmental Relative Moldiness Index (ERMI) scale to standardize the assessment of the mold contamination in US homes (Vesper et al. 2007).

The ERMI metric is based on the analysis of 36 widely distributed indicator molds: 26 group 1 molds, which are associated with water damage in homes, and 10 group 2 molds, which primarily enter the home from the outside environment (Vesper 2011). The ERMI value for a home is calculated, as shown in Eq. 1. The summed common logs of the concentrations of the group 2 molds (s_2) were subtracted from the summed common logs of the concentrations of group 1 molds (s_1) to produce the ERMI value (Vesper et al. 2007). This metric was

$$\text{ERMI} = \sum_{i=1}^{26} \log_{10}(s1i) - \sum_{j=1}^{10} \log_{10}(s2j) \tag{1}$$

previously applied in a pilot study of molds in settled dust from inside eastern Puerto Rican homes (Bolaños-Rosero et al. 2013).

The objective of this study was to determine the impact of Hurricane Maria on fungal levels in homes located in the low-income community (69.8% living below the federal poverty level) of Piñones located in the Loíza municipality on the northeastern coast of Puerto Rico. The residents (about 2500) are predominantly black Hispanic with 35.8% of the population under 18 years of age in 2010, the latest available data (US Census 2010). Three natural barriers (a river, a large mangrove forest, and the ocean) separate Piñones from the rest of the municipality, leading to greater environmental exposures and reduced healthcare access (closest clinic is about 15 km away) (García-Rivera et al. 2017). Understanding the impact of events like hurricanes on this environmental justice community is necessary to correct housing disparities and increase resiliency to natural disasters.

Material and methods

Home selection

Because of the sensitive conditions after the event, participants in this research were selected by means of their interest and their availability to participate in the study. The volunteered participant' households were categorized based on their proximity to the local bodies of water, by considering that the exposure to them could be a factor affecting both the initial impact and then the long-term effect in the sampled households. Based on this, we divided that sampled households into five different regions (region 1, $n = 6$; region 2, $n = 5$; region 3, $n = 2$; region 4, $n = 5$; and region

5, $n = 2$) within the community of Piñones (Fig. 1). This study is cross-temporal, taking measurements for both years 2018 and 2019. For the second year (2019), some families declined to continue as part of the study.

Because of IRB requirements, participants in the study were selected by their interest to participate. In a post hoc evaluation of the participant household locations, we decided to group these households based on their proximity regarding location. Households for each of the five regions of analysis are located in proximity to each other by less than 100 m, and each of these regions is separated by 300 m or more. The assumption for the post hoc grouping was that proximity/separation of households to each other could impact potential similarities/differences for the studied households.

This study was approved by the IRB Committee of the Medical Sciences Campus, UPR (A9830117) and the CIPSHI of the University of Puerto Rico-Rio Piedras (1617–033).

Home assessments

The studied area was subject to the impact of both Hurricanes Irma and Maria during 2017. The area was not reported as flooded, but households were subject to the impact of rain and winds, and households suffered damaged ranking from minor (water entering through windows) to major (structural damage to roof and/or walls).
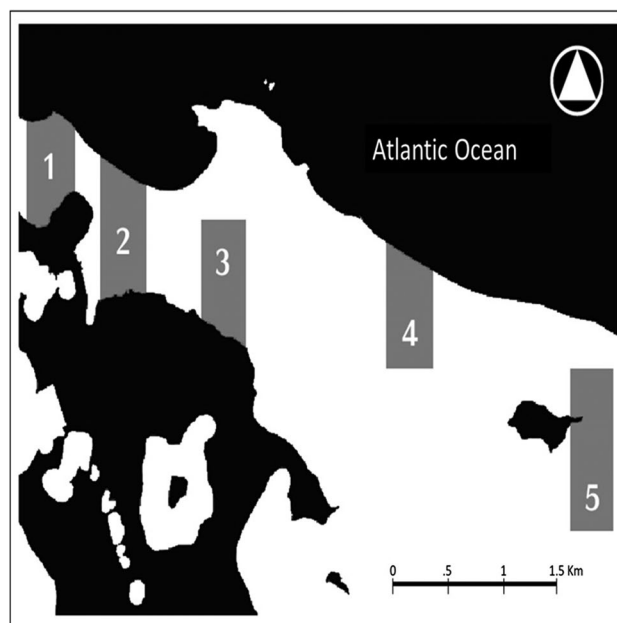

Fig. 1 Sampling locations in regions 1 to 5 in Piñones. The gray areas of the map show the footprint of the regions in Piñones community and each region's proximity to water, shown as the black areas

In order to document the conditions of the households, we identified the owner of the participant homes, and we assed via interviewing basic information regarding exposure to damage, repairs to the households after the event, material characteristics of the households, type of ventilation of the spaces, and presence of pets (see Table 1). These variables were collected as literature has shown potential connections between them and the occurrence of fungi in households.

Dust sampling

Dust samples were collected in different areas within the living room and bedroom, (e.g., door frames, bookshelves, and windowsills), using a clean electrostatic cloth (EC) (Swiffer™, Proctor and Gamble, Cincinnati, OH). Once the cloths were completely impregnated with dust (maybe describe here what means "completely impregnated with dust"); it was placed in sealable plastic bags (Ziploc®, SC Johnson, Racine, WI). The cloths that contained the dust samples were placed in a cooler (4 °C) and returned to the laboratory where they were stored at −80 °C until analyzed.

Quantitative PCR (qPCR) analyses of dust samples

The EC dust was recovered, as previously described (Cox et al. 2017). Five milligrams of sieved (pore 300 μm) dust from each sample were added to a 2-ml extraction tube containing 0.3 g of glass beads, as previously described (Haugland et al. 2004). Each EC or vacuum dust sample was spiked with 1×10^6 conidia of *Geotrichum candidum* at the time of extraction as an internal reference to ensure that the extraction and purification were performed correctly (Haugland et al. 2004). A bead beater (BioSpec Products, Bartlesville, OK) was used to shake each extraction tube at 5000 rpm for 1 min to release the DNA from the cells. The DNA was then purified using the DNA-EZ extraction kit (GeneRite, Monmouth Junction, NJ), following the manufacturer's instructions.

Each of the 36 ERMI molds was quantified in each extract with qPCR assays (Haugland et al. 2002). The standard qPCR assay contained 1 μl of a mixture of forward and reverse primers at 25 μM each, 12.5 μl of "Universal Master Mix" (Applied Biosystems Inc., Foster City, CA), 2.5 μl of 2 mg ml^{-1} fraction V bovine serum albumin (Sigma

Chemical, St. Louis, MO), 2.5 μl of a 400 nM TaqMan probe (Applied Biosystems Inc., Foster City, CA), and 2.5 μl of DNA-free water (Cepheid, Sunnyvale, CA). Five microliters of the DNA extract from the sample and this mix were combined. Reactions were performed with thermal cycling conditions consisting of 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C for template denaturation, and 1 min at 60 °C for probe and primer annealing and primer extension, and 5 mg from each of the sieved-dust samples was analyzed by a commercial laboratory that performs the ERMI analysis (Mycometrics LLC, Monmouth Junction, NJ).

Analyses

The average concentration of each of the 36-ERMI molds in the dust samples from 2018 to 2019 were compared using the Wilcoxon rank sum test, corrected for multiple comparisons using the Holm–Bonferroni test. The average sum of the logs of the group 1 molds, sum of the logs of the group 2 molds, or ERMI values were compared between sampling years using the Student's *t*-test (Table 2).

The diversity of mold populations in the homes in the study was determined with the Shannon index of equitability (Shannon & Weaver 1963). The diversity boxplots were built using R's ggplot2 package (Wickham 2009) from a species table and two corresponding files, taxonomy and metadata, as a phyloseq object, and alpha diversity was plotted with the "plot_richness" function (McMurdie & Holmes 2013) using year and region as sample groupings. Statistical tests for SDI values by year and region were done using libraries "ggpubr" and "ggplot2" in R (Kassambara 2018) by calculating pairwise differences between region level means with Tukey's multiple comparisons of means tests.

Results

Our project calculated the comparisons between the average population of each of the 36-ERMI molds in the homes in the community of Piñones in 2018 and 2019. Neither the sum of the logs of the group 1 molds nor the ERMI values changed significantly between 2018 and 2019. However, the sum of the logs of the group 2 molds did increase significantly in

Table 1 Characteristics of homes studied in the five regions of the Piñones community, shown as number of homes with the characteristic per total number of homes in that region. *AC* air-conditioning

Region	Water damage	Material	Ventilation	Dog
1	0/5	5 Cement	5 Natural	0/5
2	2/5	4 Cement; 1 Wood	5 Natural	1/5
3	1/2	2 Wood	2 Natural	0/2
4	0/5	5 Cement	4 Natural; 1 full AC	2/5
5	1/2	1 Cement; 1 vinyl	2 Natural	2/2

2019 compared to 2018 (7.8 vs 4.8, respectively) (Table 2). There were two species of group 2 molds, *Alternaria alternata* ($p < 0.001$) and *Cladosporium cladosporioides* Type 1 ($p < 0.001$), whose concentrations indoors were significantly greater in 2019 than 2018 (Table 2).

Next, the change in the diversity of the mold populations in the homes in each of the regions were compared

between 2018 and 2019 by plotting the Shannon Diversity Index (SDI) value (Fig. 2). The homes in regions 1 and 4 were spared of water damage from Hurricane Maria. Two of the homes in region 2 received some water damage. The SDI values for homes in regions 1, 2, and 4 changed a little between 2018 and 2019 (Fig. 2). In region 3, the SDI value was the highest of any region in 2018 and only decreased

Table 2 Average (AVG) concentration in cell equivalents per mg dust (CE/mg dust) of each of the 36 Environmental Relative Moldiness Index (ERMI) molds in dust samples collected in 2018 and 2019 in Piñones homes compared using the Wilcoxon rank sum test (*p*-value), corrected for multiple comparisons using the Holms–Bonferroni test. Average sum of the logs of the group 1 or group 2 molds or ERMI values compared using the Student's *t*-test (*p*-value). (*NM* no meaning; significant differences are bolded.)

Year	2018 AVG	2019 AVG	*p*-value
Group 1 molds			
Aspergillus flavus	12	29	0.136
Aspergillus fumigatus	2	3	0.211
Aspergillus niger	156	357	0.008
Aspergillus ochraceus	2	13	0.017
Aspergillus penicillioides	42,363	22,068	0.459
Aspergillus restrictus	94	31	0.095
Aspergillus sclerotiorum	68	35	0.479
Aspergillus sydowii	322	326	0.981
Aspergillus unguis	13	19	0.528
Aspergillus versicolor	668	164	0.384
Aureobasidium pullulans	170	87	0.321
Chaetomium globosum	73	111	0.534
Cladosporium sphaerospermum	10,082	468	0.388
Eurotium amstelodami	178	300	0.391
Paecilomyces variotii	16	14	0.882
Penicillium brevicompactum	3	12	0.064
Penicillium corylophilum	1	1	NM
Penicillium crustosum	7	615	0.247
Penicillium purpurogenum	2	9	0.073
Penicillium spinulosum	1	1	NM
Penicillium variabile	30	12	0.347
Scopulariopsis brevicaulis	4	8	0.292
Scopulariopsis chartarum	91	32	0.443
Stachybotrys chartarum	2	2	0.410
Trichoderma viride	18	9	0.416
Wallemia sebi	2318	2346	0.992
Sum logs group 1	28.9	30.7	0.45
Group 2 molds			
Acremonium strictum	1	3	0.032
Alternaria alternata	5	17	**< 0.001**
Aspergillus ustus	3	5	0.169
Cladosporium cladosporioides 1	508	1530	**≤ 0.001**
Cladosporium cladosporioides 2	3	3	0.928
Cladosporium herbarum	1	2	0.076
Epicoccum nigrum	1	1	0.254
Mucor group	14	50	0.184
Penicillium chrysogenum 2	1	1	0.324
Rhizopus stolonifer	4	41	0.068
Sum logs group 2	**4.8**	**7.8**	**< 0.01**
ERMI	24.2	22.9	0.52

Fig. 2 Shannon Diversity Index (SDI) values for the five regions in Piñones, in 2018 and 2019. Tukey HSD (honestly significant difference) tests were applied to the mean values of each region; none of the pairwise comparisons were significantly different after *p*-adjustment (see suppl table 1)
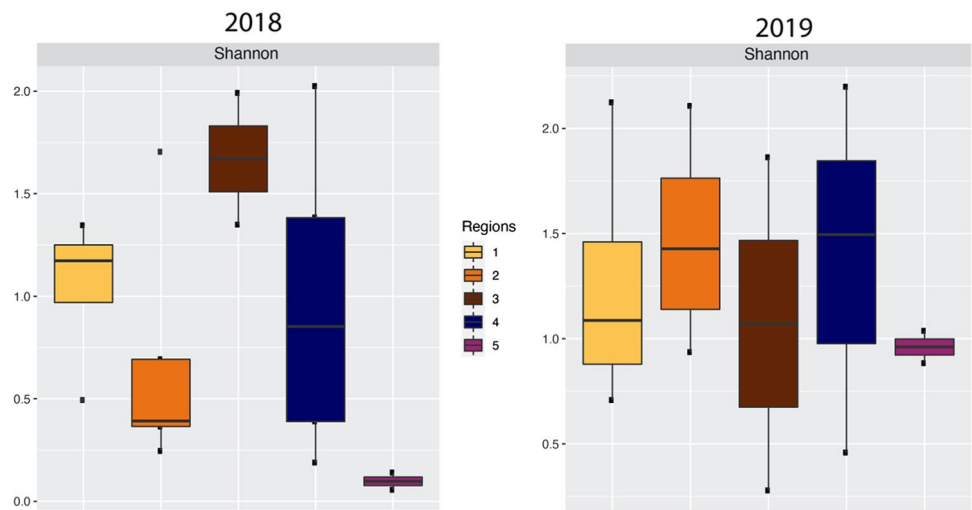
slightly in 2019. In region 5, homes had the lowest SDI value of any of the regions in 2018, but, in 2019, the SDI value for region 5 homes increased nearly to levels and then found in homes in regions 1, 2, and 4, although average values between sectors were non-significant (Fig. 2).

Because we do not have a previous set of measurements either of this sector and/or of the participating households, we compared our results to a previous study of households located on eastern Puerto Rico (Bolaños-Rosero et al. 2013) in which also mold levels were assessed (Fig. 3). We found that the average SDI value in the Piñones community was significantly lower in 2018 than the SDI value in the eastern Puerto Rico homes in 2013 (*p*-adjusted value = 0.004) (Fig. 3). But, by 2019, the average SDI value in the Piñones community was near the average SDI value measured in eastern Puerto Rico homes in 2013 (*p*-adjusted value = 0.401) except for those households that have reported damage and little or no remediation (Fig. 3). The log of the average yearly outdoor mold-spore counts and their standard deviations (SD) in the greater San Juan area of Puerto Rico in 2017, 2018, and 2019 showed significant higher levels of outdoor mold spore counts in 2018 a year after Hurricane Maria (Table 3).
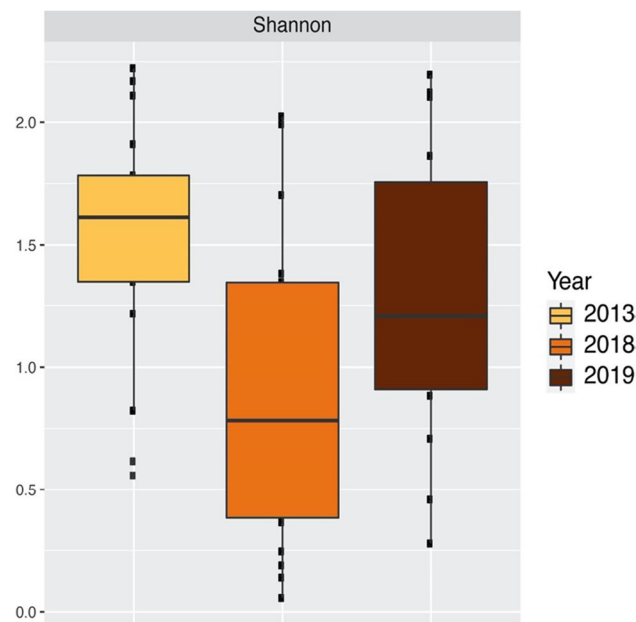
Discussion

Homes in regions 1, 2, and 4 of the in Piñones community were generally spared major water damage. Therefore, the SDI values for the homes in these regions were little changed between 2018 and 2019. However, wind and heavy rain caused a tree to fall through the roof of one home in region 5, resulting in low SDI values. The other home in region 5, which was next door and occupied by the same extended family of people and dogs, resulted in cross-contamination between the homes. Fortunately, these homes were made of cement or vinyl. Once the roof was repaired and the furnishings removed or cleaned, the

Fig. 3 Shannon Diversity Index (SDI) values for the homes in eastern Puerto Rico sampled in 2013 and the average of the five regions in Piñones in 2018 and in 2019. A Tukey multiple comparisons of means test was applied to the SDI mean values and showed that there were significant differences between 2013 and 2018 (*p*-adjusted = 0.004)

Table 3 Log of the average yearly outdoor mold-spore counts and their standard deviations (SD) in the greater San Juan area of Puerto Rico in 2017, 2018, and 2019. Averages that are followed by the same letter are not significantly different

Year	Average	SD	Tukey
2017	4.6812	0.1417	**a**
2018	4.8593	0.1781	**b**
2019	4.7335	0.0895	**a**

SDI value returned to a level comparable to regions 1, 2, and 4.

One of the homes in region 3 had obvious water damage, but both homes in region 3 were made of wood. As opposed to cement or vinyl, wood is much more susceptible to mold growth when it gets wet, and more difficult to dry-out. The SDI value for these region 3 homes did not improve between 2018 and 2019. This finding suggests that wood homes should be targeted for rapid repair and remediation in the wake of hurricanes. For these reasons, Ma and Smith (2020) suggested that "Disaster preparedness policies should raise structural standards for low-income housing to reduce risks of severe damage."

In addition to impacts on housing, Hurricane Maria severely damaged much of Puerto Rico's vegetation. Scholl et al. (2021) reported major defoliation due to Hurricane Maria. Hall et al. (2020) utilized field and remote sensing data to determine that 23% of island's pre-hurricane forest had been destroyed. The category 4 Hurricane Maria killed twice as many trees as the category 3 Hurricane Hugo in 1989, and Maria broke 2- to 12-fold more stems than Hugo or George in 1998, also a category 3 hurricane (Uriarte et al. 2019). These major impacts on the vegetation appear to have released many phylloplane mold spores, i.e., molds that live on leaves, into the air, which led to the high outdoor mold counts in 2018 in San Juan AAAAI located 20.1 km from Piñones under the 30-km region reasonable estimation established (Katelaris et al. 2004) area (Fig. 3). However, by 2019, the outdoor mold-spore counts had returned to their pre-hurricane levels (Table 3).

Cladosporium cladosporioides and *Alternaria alternata* are well-known phylloplane molds (Miller 1992; Nix-Stohr et al. 2008; Hedayati et al. 2009). The increased level of these two molds was observed indoors in 2019 in the qPCR data. A group 2 mold like *Alternaria alternata* is an important source of allergens and may have implications for asthma prevalence (Grewling et al. 2020).

Although we do not have post-hurricane estimates of asthma in Piñones, the islands of St. Thomas, St. John, and the US Virgin Islands reported that there was a significant increase in patients being seen for diabetes-related and respiratory complaints, especially asthma (Chowdhury et al. 2019). It may take years to determine the impact of Hurricane Maria on asthma prevalence in Piñones.

Respiratory illnesses like asthma are expected to increase because of increases in exposure to pollen and mold resulting from climate change (D'Amato et al. 2020). Previously, urbanization was shown to be associated with reduced microbial outdoor exposure and increased contact with housing materials, which led to increased house and skin-mold diversity (McCall et al. 2020). It appears that climatic and urbanization changes may alter the prevalence of diseases associated with mold exposures.

There are many limitations to our study, including the low number of homes sampled. Some of the problems with recruitment arose because of the earthquakes that disrupted our recruiting efforts. In addition, some families did not allow the resampling of their homes in 2019. We also quantified a set of 36 molds as defined by ERMI under the assumption that the presence of these molds might help to assess the relationship between the presence of these molds, exposure, and possible impact on the respiratory health of these household inhabitants (Vesper & Wymer 2016).

However, and despite these limitations, our findings point out to the role that both house repairment and physical location might play in prolonged mold impacts after the exposure to severe weather events like hurricanes. Prompt action to repair major water damage seems to be relevant for quickly returning homes to typical mold levels in these households, and thus be a crucial factor for the short term improvement of indoor air quality after severe water exposure.

Conclusion

After major atmospheric events such as hurricanes, fungal populations inside of the affected homes can have serious impacts into its microbial communities. Our results point out to the role that both location of the households and timing for damage repair play in the effects of indoor fungi after a hurricane. Additionally, the endurance of that effect through time and the possible long-term impact on the health of their inhabitants are suggested.

Declarations

Ethics approval This study was approved by the Ethics Committee of the University of Puerto Rico, Rio Piedras Campus (*June 14, 2021, Fungi, asthma and built environment: A pilot study IRB A9830117*).

Consent to participate Informed consent was obtained from owners of sampled homes.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

Disclaimer The US Environmental Protection Agency (EPA) through its Office of Research and Development collaborated in the research described here. It has been subjected to the Agency's peer review and has been approved as an EPA publication. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use. The findings and the conclusions in this report are those of the authors and do not necessarily represent the views of the US EPA.

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