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Original Article

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Application of the Environmental Relative Moldiness Index in Indoor Marijuana Grow Operations

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Abstract

Objectives: Indoor marijuana grow operations (IMGOs) are increasing due to legalization of recreational and medicinal cannabis at the state level. However, the potential exposures of IMGO workers have not been well studied. Mold exposure has been identified as a major occupational health concern. Mold-specific quantitative polymerase chain reaction (MSQPCR) can provide quantitative exposure data for fungi at the species level. The purpose of this study was to characterize the airborne fungal burden using MSQPCR and to evaluate the applicability of an airborne Environmental Relative Moldiness Index (ERMI) in IMGOs.

Methods: Air and dust samples were collected inside and outside the IMGOs and then analyzed via MSQPCR. These data were then used to calculate IMGO-specific ERMI scores. Culturable air samples were collected on agar plates and analyzed via microscopy. Differences were evaluated between indoor and outdoor concentrations, as well as between air and dust samples. The agreement between MSQPCR and culture-based methods was also evaluated.

Results: Based on the geometric means for non-zero values of each fungal species across all IMGOs, the total airborne concentration was approximately 9100 spore equivalent (SE) m^{-3} with an interquartile range (IQR) of 222 SE m^{-3}. The indoor/outdoor ratio of geometric means across all 36 species per IMGO ranged from 0.4 to 6.2. Significantly higher indoor concentrations of fungal species, including *Aspergillus* spp., were observed. An average airborne ERMI score of 7 (IQR = 7.6) indicated a relatively high burden of mold across a majority of operations. The ERMI scores were driven by the high

concentrations of Group 1 species with a mean of 15.8 and an IQR of 13. There were 63 additional species identified in the culturable air samples not included in the ERMI.
Conclusions: High concentrations of airborne fungi were identified in IMGOs. Our evaluation of the ERMI based on MSQPCR as a rapid diagnostic and risk assessment tool for industrial hygienists in the IMGO setting is equivocal. ERMI did not identify all relevant fungal species associated with this specific occupational environment. We identified several issues with using the ERMI calculation. At this time, the catalog of fungal species needs to optimized for the occupational setting to ensure adequate coverage, especially for those species expected to be found in this burgeoning industry. Further research is necessary to elucidate the link between the ERMI score of airborne samples, worker exposure and health effects in grows to generate an acceptable index score for use in occupational exposure assessments.

Keywords: cannabis; Environmental Relative Moldiness Index (ERMI); indoor marijuana grow operations (IMGOs); mold; MSQPCR

Introduction

Since 2012, the USA has experienced a trend in legalization of recreational cannabis. Currently, 33 states have legalized either recreational or medicinal cannabis use (Governing, 2018; Orens *et al.*, 2018). To meet the rapid increases in cannabis sales and commercialization nationally, the industry has had to increase the infrastructure and develop technology for mass cultivation. This includes the construction of greenhouses, commonly referred to as indoor marijuana grow operations (IMGOs), to cultivate plants and create economies of scale to meet demand. As in other agricultural operations, there are considerable occupational health risks that, as of yet, have not been well described or investigated in this emerging field.

Inhalation exposures are likely to be a major concern among workers in IMGOs (Martyny *et al.*, 2013). Through generation of an ideal indoor climate (i.e. subtropical), growers can achieve a higher yield of plants with increased concentrations of delta-9-tetrahydrocannabinol (THC), the pharmacologically active metabolite in cannabis (Ghosh *et al.*, 2017). Such environments are at a high risk for excessive moisture leading to fungal growth and contamination (Johnson and Miller, 2012). Specifically, elevated levels of airborne mold spores were observed as a primary health concern during plant disturbance and removal by law enforcement prior to widespread legalization (Martyny *et al.*, 2013). In a variety of other occupational settings, high levels of airborne mold and subsequent worker exposure have resulted in non-allergenic and allergenic respiratory diseases, including exacerbation of asthma and development of hypersensitivity pneumonitis (Douwes *et al.*, 2003).

While the health effects of occupational fungi exposure are well documented, there are no established dose–response relationships, particularly for respiratory endpoints. Part of this limitation arises from traditional mold sampling methods, which include total spore counts via slit impactor collection and culture enumeration via growth media collection (Niemeier *et al.*, 2006). As noted by the World Health Organization and Institute of Medicine, counting and enumeration have serious limitations for use in human health risk assessments and exposure assessments (Institute of Medicine (US) Committee on Damp Indoor Spaces and Health, 2004; Heseltine *et al.*, 2009). These limitations include lack of universally standardized methods, reproducibility, and selectivity (Godish and Godish, 2007; Crawford *et al.*, 2009; Pityn and Anderson, 2013). Further, the measurement of culturable fungi and fungal spores is impeded by relatively short sampling periods. While shorter air sampling times are required to reduce overloading of culture and impaction plates, significant shifts in microorganism abundance and diversity have been observed in relatively short sampling periods (i.e. work shift) (Spicer and Gangloff, 2007; Fierer *et al.*, 2008). Hence, these shorter sample times associated with traditional spore traps and culture-based methods will not account for dynamic changes in fungal species over longer durations. Following, these methods may result in the underestimation of fungal abundance and diversity.

Molecular-based analytical methods offer significant advantages over traditional mold measurements. Specifically, mold-specific quantitative polymerase chain reaction (MSQPCR) provides a highly sensitive and rapid alternative for quantitative measurement of fungal species. This method, developed by the United States Environmental Protection Agency, targets the internal transcribed spacer region of fungal DNA to provide species-specific concentrations (Haugland and Vesper, 2002). MSQPCR is applicable to filter-based air samples and affords longer sample times (Meklin *et al.*,

2004). While culture-based methods can be applied to filter-based samples, MSQPCR relies on DNA for species identification as opposed to selective and differential techniques. As such, MSQPCR allows for a wider array of fungal species to be identified. In comparison, culture-based methods only allow for detection of viable species that can proliferate on the appropriate growth media.

The utility of MSQPCR is demonstrated in evaluating indoor environments for comparative metrics of fungal loading. Applying MSQPCR to settled dust samples, researchers identified two different groups of fungal species associated with water-damaged homes (Group 1; 26 species) and outdoor sources (Group 2; 10 species) (Meklin *et al.*, 2004). By comparing these two groups of indicator species, the relative contribution of fungi from water-damage source or outdoor sources can be estimated and used to describe the fungal burden. Subsequently, the Environmental Relative Moldiness Index (ERMI) was developed and applied to settled dust samples collected across a large subset of US homes (Meklin *et al.*, 2007; Vesper *et al.*, 2007). This index ranges from −10 to 20+ where higher scores are associated with higher mold burden. Previous research has demonstrated that adults and children with asthma tend to live in homes with higher ERMI scores compared with homes of participants without chronic respiratory disease (Blanc *et al.*, 2013; Vesper *et al.*, 2015, 2016; Vesper and Wymer, 2016). When the ERMI was tested outside the USA, varying results of occurrence of fungal species were observed. These differences were most likely due to climatic conditions and/or inoculum sources (e.g. plant sources) (Méheust *et al.*, 2013) which suggests that other species may be more relevant depending the environment. The utility of ERMI outside of residential settings is unknown. We sought to understand if application of the ERMI may provide a novel risk assessment framework for occupational hygienists and health practitioners assessing fungal burden in IMGOs.

In 2012, Colorado was the first state to legalize cultivation, purchase, and use of cannabis for recreational purposes (Maier *et al.*, 2017). A report released in 2018 estimated that from 2015 to 2017, overall cannabis sales in Colorado increased from $996 million to $1.5 billion (increase of 51.6%). Currently, Colorado has approximately 2900 licensed marijuana businesses and over 41 000 individuals licensed to work in the industry. Accordingly, Colorado is an optimal location to study potential occupational exposure concerns associated with the emerging cannabis industry. The goals of this study were the following: (i) characterize the burden of airborne fungi in IMGOs using MSQPCR for the 36 species included in the ERMI; (ii) calculate the fungal burden of IMGOs according to the ERMI; (iii) compare MSQPCR results to culture-based methods for species presence and concentration within the IMGOs; and (iv) evaluate the utility of the ERMI in air samples versus settled dust. Given the difference in indoor environments between occupational and household settings and use of a distinct environmental matrix, our objective was to determine if ERMI demonstrates utility in assessing the fungal burden of IMGOs. The conditions and sources of mold growth and contamination within these indoor operations were anticipated to be substantially different than those observed in residential settings. As such, we hypothesized that the ERMI would not be a reliable indicator of fungal contamination and that a new index of relevant fungal species specific to IMGOs would need to be developed.

Methods

Study sites

This study was conducted over a 1-year period starting in January 2011. In cooperation with the Colorado Drug Investigators Association, 22 illegal IMGOs throughout the Northern Front Range of Colorado (along the Interstate 25 corridor ranging from the Denver Metro to Fort Collins) were identified. Because data collection commenced in 2011 and was completed in January 2012, only medicinal cannabis was legal during this study period. IMGOs enrolled in this study were considered illegal because they did not possess a license from a local authority and/or the number of plants cultivated exceeded the 36 plant limit. The number of plants per IMGO in this study ranged from 11 to 670 cultivated in hydroponic and soil-based systems. These operations also controlled day–night periodicity and carbon dioxide levels to enhance increased plant yield of THC. After the operation was entered and secured by law enforcement, researchers conducted a walkthrough and inspection to determine optimal air sampling locations inside the grow room and outside the building structure for comparison and source apportionment (indoor/outdoor concentration ratio). Since these operations were illegal, the number of workers and their specific tasks in each IMGO were not determined. General tasks in these operations encompassed all aspects of cultivation ranging from transplanting, cloning, trimming, harvesting, and drying of plants.

Sample collection and analysis for culturable fungi and fungal DNA

Culturable air samples were collected at 28.3 l min^{-1} using a 400-hole impactor (Standard BioStage, SKC, Inc., Eighty-Four, PA, USA) connected to a QuickTake 30 (SKC, Inc., Eighty-Four, PA, USA). A total of four

impactor samples were collected at each location (including outdoor air) within a study site. Two samples were collected on malt extract agar (MEA) and two were collected on dichloran glycerol 18% agar (DG-18) to improve the resolution and isolation of culturable fungi. MEA was selected as general-purpose media for isolating an array of environmental and pathogenic fungi. In conjunction with MEA, DG-18 plates were deployed to foster growth of the slower growing species (i.e. by inhibiting fast growing species). Samples were collected in duplicate in each location for 2 min. All agar plates were prepared by EMSL Laboratories (Cinnaminson, NJ, USA). Full details and results for the culture-based methods are available in Martyny *et al.* (2013).

To collect airborne fungal DNA, MSQPCR samples were collected at 15 l min^{-1} using 25-mm styrene cassettes loaded with polycarbonate filters. MSQPCR samples were collected for approximately 60 min. All pumps were pre- and postcalibrated using a primary standard. Differences <5% were considered acceptable. Settled dust samples were collected using Duststream collectors attached to a Sanitaire SC3683 vacuum cleaner (Electrolux, Peoria, IL, USA) as previously described (Van Dyke *et al.*, 2012). Briefly, settled dust samples were collected by vacuuming a 2 m^2 area in the grow room or adjacent to the grow room.

All samples were shipped by overnight delivery to an American Industrial Hygiene Association accredited laboratory for microscopy and PCR analysis. Here, analyses were conducted using proprietary methods based on modifications to standard techniques. In general, MEA and DG-18 samples were incubated and fungal colonies were counted under a stereomicroscope. Fungal species were identified by comparison of morphological features and characteristics to mycology keys. In accordance with the EPA Patent, MSQPCR was conducted using forward and reverse primers for the 36 species included in the ERMI calculation (Haugland and Vesper, 2002). Briefly, genomic DNA was extracted from samples using standard methods that mechanically disrupt the cell. Genomic DNA from each sample was added to the reaction assay mixture containing species-specific forward primers, reverse primers, and probes based on the internal transcribed spacer regions of the nuclear ribosomal DNA found in fungi. All primers and probes used in the assay were reported previously (Haugland and Vesper, 2002).

ERMI calculation

Reported MSQPCR results in the form of spore equivalent (SE) counts for the 36 different species were used to calculate ERMI values as described in equation (1) (Vesper *et al.*, 2007).

$$\mathrm{ERMI} = \sum_{i=1}^{26} \log_{10}(S_{1i}) - \sum_{j=1}^{10} \log_{10}(S_{2j}) \qquad (1)$$

SE counts of each Group 1 (water damage) species and Group 2 (non-water damage) were converted to log values and summed. The sum of the logs of Group 2 species (S2) was then subtracted from the sum of the logs of Group 1 species (S1) to obtain the ERMI score.

Statistical analysis

Data were normalized to the amount of air sampled and dust vacuumed for respective sample types collected at each IMGO. For culture-based methods, data were reported in colony forming units per cubic meter (CFU m^{-3}). For MSQPCR-based methods, data were reported as SEs per mg of dust for settled dust samples and SE m^{-3} for air samples. Data were cleaned using tidyverse packages in R. Outdoor and indoor samples were separated for analyses. The MSQPCR data were not normally distributed, varying by orders of magnitude depending on species and location. Descriptive statistics of fungal loadings are presented as geometric mean, geometric standard deviation, and the interquartile range (IQR). Data sets contained non-detect values, or 0 SE m^{-3}, for different species across each IMGO. As a result, only non-zero values were included in the calculation of geometric means and geometric standard deviations.

Indoor/outdoor comparison

Heat maps were generated for both indoor and outdoor airborne MSQPCR samples using R package superheat Version 0.1.0 and organized by IMGO. Given the wide variation in the data, the heat maps were smoothed by first centering the middle value of each column (IMGO) and then scaling the color gradient to differentiate various counts of species. Correlation matrices comparing each species were generated using Spearman's rank correlation coefficient. Matrices were then used to generate correlograms using the R package corrplot Version 0.84. We conducted a two-sided paired Wilcoxon signed-rank test to determine if there were statistically significant differences in indoor and outdoor airborne fungal concentrations at each IMGO.

Methods comparison

To determine if the culture-based methods led to differences in species detection compared with MSQPCR for the 36 species included in the ERMI, we created a binary indicator (Y/N) for each of the species based on

detection by each method, and summed results across all facilities in the study. We then implemented McNemar's test for each species in ERMI to evaluate the agreement between detection methods. To determine the extent of detectable species that were not included in the ERMI, we evaluated the presence or absence of seventy additional species that were able to be detected through culture-based methods and summed presence of each of these indoor species (Y/N) across the IMGOs included in the analysis.

Dust/airborne comparison

To determine if there were differences in indoor fungal concentrations by sampling method/matrix, we compared the indoor dust levels collected through vacuuming with the indoor air samples collected by filter-based methods also using a two-sided paired Wilcoxon signed-rank test.

The Wilcoxon signed-rank test and the McNemar's test were conducted in SAS 9.4.

Results

A total of 24 air MSQPCR samples were collected inside 22 different IMGOs. Grows were primarily contained inside single-family residences (n = 20) while one operation was in an office building (IMGO #13) and another located in a commercial warehouse that was producing 670 plants (almost 20× the number of plants allowed at the time). The highest number of plants in a residential grow was 240 plants. Three MSQPCR samples were collected in IMGO #13 since this was a commercial office building with multiple units dedicated for growing plants (Supplementary Table S1, available at *Annals of Work Exposures and Health* online).

Culture

The results presented in this section are recapitulated from a previously published companion study (Martyny *et al.*, 2013). The mean percent relative humidity (% RH) in these operations was 51% (range: 23–94%). A total of 228 culture-based air samples were collected inside the IMGOs (MEA: 114; DG-18: 114). Briefly, the median count of culturable fungi was >1625 CFU m^{-3} (range: 72 to >10 836 CFU m^{-3}). When compared with outside samples (n = 92), concentrations of culturable fungi were, in general, higher inside the operations. The median ratio of indoor/outdoor concentrations was 2.1 (range: 0.3–16.5). Hence, based on culture-based methods, dominant shifts in concentrations and fungal taxa were observed between outside and inside samples. A secondary analysis of these data was performed to evaluate the use of MSQPCR to measure fungi in air samples (presented below).

MSQPCR

The concentrations of 36 fungal species across all IMGOs are summarized in Table 1 by location (indoor and outdoor air) and matrix (air versus settled dust). Indoor air MSQPCR results are based on samples collected across 22 different IMGOs (as stated above). However, those results from dust and outdoor air sampling are reported from only 4 and 17 locations, respectively. This imbalance in sample sizes was due to limitations (discussed below), which consequently, limited comparisons between locations and matrices across all IMGOs. Further, for several species there was only one observation across the entire study (e.g. *Aspergillus flavus* in indoor air). As a result, the values for these species were reported as a maximum count only and were not included in the summary of geometric means.

Indoor air

The concentration of airborne fungal species varied widely in the indoor air of IMGOs. Maximum species counts ranged from 0 (*Aspergillus sydowii*, *Penicillium corylophilum*, and *Penicillium spinulosum*) to 64 343 (*Trichoderma viride*) SE m^{-3} (Table 1). Based on the geometric means for non-zero values of each fungal species across all IMGOs, the total airborne concentration was approximately 9100 SE m^{-3}. The IQR of geometric means was 222 SE m^{-3} (25th and 75th percentiles: 63 and 273 SE m^{-3}). Alternatively, to highlight the extreme range of the fungal species observed across IMGOs, the range of geometric means was 7 SE m^{-3} (*Alternaria alternata*) to 44 110 SE m^{-3} (*Penicillium variable*). Despite high concentrations of *A. flavus* (1088 SE m^{-3}), *Penicillium crustosum* (Group 2; 8133 SE m^{-3}), and *P. variable* (44 110 SE m^{-3}), these species were observed one time across the entire study. Further, three species (i.e. *A. sydowii*, *P. corylophilum*, and *P. spinulosum*) were not detected in indoor air of IMGOs.

Each IMGO was represented by a unique fingerprint of fungal species with differences between taxa that spanned several orders of magnitude. The distribution of airborne fungal species (SE m^{-3}) within each IMGO is shown in Fig. 1a. In IMGO 12, high concentrations of fungal species with allergenic properties were observed, including *Aspergillus niger* (24 622 SE m^{-3}), *Aspergillus sclerotiorum* (26 084 SE m^{-3}), *Chaetomium globosum* (40 876 SE m^{-3}), *Paecilomyces variotii* (31 995 SE m^{-3}), *P. variable* (44 110 SE m^{-3}), *Scopulariopsis chartarum*

Table 1. Summary of MSQPCR results (SE m^{-3}) by sample location and matrix.

Species name	Indoor air (n = 24)			Indoor dust (n = 4)			Outdoor air (n = 17)		
	Max. count	GM[a]	GSD[b]	Max. count	GM[a]	GSD[b]	Max. count	GM[a]	GSD[b]
Aspergillus flavus	1088	-	-	6	-	-	37	-	-
Aspergillus fumigatus	1127	122	6.3	106	17.8	12.4	412	148	4.0
Aspergillus niger	24 622	94	6.7	135	81.3	2.0	264	72	8.5
Aspergillus ochraceus	156	80	2.6	761	-	-	ND	ND	ND
Aspergillus penicillioides	1177	342	2.2	ND	ND	ND	1541	325	18.8
Aspergillus restrictus	3282	599	4.6	721	-	-	2180	2180	6.5
Aspergillus sclerotiorum	26 084	155	12.8	31	-	-	91	56	3.9
Aspergillus sydowii	ND	ND	ND	1554	-	-	ND	ND	ND
Aspergillus unguis	169	-	-	ND	ND	ND	52	37	3.3
Aspergillus versicolor	28 426	1792	12.4	83	83.0	0	371	371	4.2
Eurotium (A.) amstelodami	6512	232	4.9	330	38.3	4.3	1381	145	15.1
Aureobasidium pullulans	5970	63	7.5	2391	547.2	5.1	1407	41	9.5
Chaetomium globosum	40 876	73	34.3	20	-	-	28	14	2.9
Cladosporium sphaerospermum	16 037	133	5.2	3035	335.1	22.6	337	75	9.5
Paecilomyces variotii	31 995	56	10.7	149	32.8	11.3	190	99	8.9
Penicillium brevicompactum	1888	273	5.3	221	46.8	7.3	1232	1158	10.4
Penicillium corylophilum	ND	ND	ND	ND	ND	ND	45	45	2.5
Penicillium crustosum (Group 2)	8133	-	-	ND	ND	ND	ND	ND	ND
Penicillium purpurogenum	2776	103	3.2	129	22.7	11.7	133	54	8.8
Penicillium spinulosum	ND	ND	ND	ND	ND	ND	ND	ND	ND
Penicillium variable	44 110	-	-	ND	ND	ND	ND	ND	ND
Scopulariopsis brevicaulis	1056	57	5.3	63	13.7	8.6	48	18	4.6
Scopulariopsis chartarum	36 166	133	12.4	41	14.3	4.4	394	49	9.3
Stachybotrys chartarum	545	67	4.8	185	185	0	37	9	2.9
Trichoderma viride	64 343	85	34.8	394	28.1	41.9	140	76	5.6
Wallemia sebi	5211	1229	6.6	780	57.9	20.7	10 859	714	48.1
Acremonium strictum	5178	2407	2.3	21	-	-	929	649	8.6
Alternaria alternata	11	7	1.7	6	-	-	142	29	5.1
Aspergillus ustus	1388	288	3.0	712	293.5	3.5	254	77	10.7
Cladosporium cladosporiodes I	3552	71	4.8	416	201.9	2.8	2105	75	10.4
Cladosporium cladosporiodes II	1002	17	4.7	2	2.0	0	101	12	4.4
Cladosporium herbarum	4728	259	5.5	412	283.1	1.4	6462	236	7.8
Epicoccum nigrum	28 407	277	9.7	484	427.2	1.2	4339	280	20.1
Mucor and *Rhizopus group*	415	11	4.4	259	34.7	9.6	6	4	2.0
Penicillium chyrsogenum	2029	32	18.6	8	-	-	83	83	2.9
Rhizopus stolonifer	24	11	1.7	11	-	-	17	10	2.8

GM, geometric mean; GSD, geometric standard deviation.

[a]The geometric mean was calculated using all non-zero values across the number of IMGOs specified.

[b]The geometric standard deviation was calculated using all non-zero values in accordance with the geometric mean.

- A geometric mean and geometric standard deviation were not calculated due to only one observation of a species in the detectable range.

[ND]Represents those species that were not detected across all number of IMGOs specified.

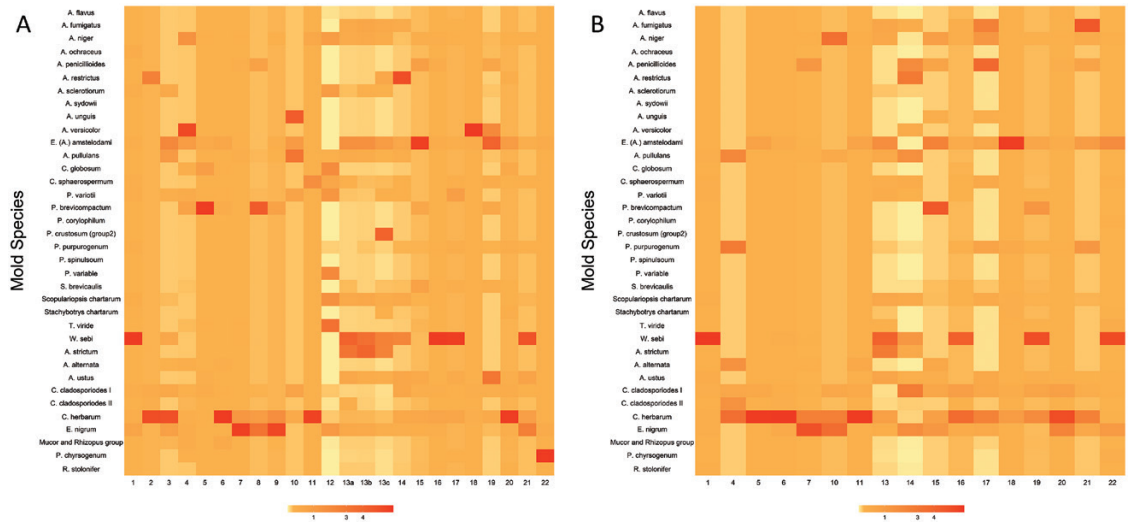

Figure 1. Heat map of the relative contribution of airborne fungal species in the indoor air (a) and outdoor air (b) of each IMGO. The color of each square is proportional to the concentration (SE m^{-3}) of the individual fungal species (i.e. dominant taxa are shown in darker red while relatively lower counts are depicted in white). The 36 species measured in this study were present in varying concentrations across locations and predominant genera were IMGO-specific.

(36 166 SE m^{-3}), and *T. viride* (64 343 SE m^{-3}). In contrast, *Aspergillus versicolor* (28 426 SE m^{-3}), which is known to be toxigenic, was the predominant taxa present in IMGO 18. The indoor air in IMGO 18 did not exhibit the level of richness as compared with the other operations, especially since *A. versicolor* was approximately two orders of magnitude higher than the five other species present. Beyond the presence of *Aspergillus* spp. across IMGOs (Fig. 1a), other abundant species included *Penicillium brevicompactum*, *Wallemia sebi*, *Acremonium strictum*, *Cladosporium herbarum*, and *Epicoccum nigrum*.

A range of positive correlations were observed across airborne fungal species in indoor air (Fig. 2a). For example, 13% of fungal species were moderately correlated (r_s = 0.4–0.66) with at least one other species. Of these, there were a total of seven correlations of at least 0.6; no correlations exceeded 0.66. Of particular interest, *Aspergillus fumigatus* was moderately correlated with nine other species, including *A. strictum* (r_s = 0.66). While ubiquitous, inhalation of *A. fumigatus* is purported to cause 90% of human respiratory infections, including invasive lung aspergillosis in immunocompromised individuals (Priyamvada *et al.*, 2017). Additionally, the following fungal species (known to cause opportunistic mycoses and allergic responses) demonstrated moderate correlations with at least nine other species (*n*): *A. niger* (10), *Eurotium amstelodami* (9), *Cladosporium sphaerospermum* (9), *Penicillium purpurogenum* (11), *Scolpulariopsis chartarum* (12), *A. strictum* (10), and *Cladosporium cladosporiodes II* (10). Last, *A. flavus*, which is a recognized as a major producer of aflatoxin (a known carcinogen), was moderately correlated with five other species. However, it is important to note that *A. flavus* was observed in only one IMGO.

In contrast to the results above, there was a larger presence of fungal species in the indoor air that demonstrated an inverse relationship—i.e. a negative correlation coefficient (Fig. 2a). Briefly, 16% of fungal species demonstrated a zero correlation while almost 30% of species were negatively correlated (ranging from r_s = –0.01 to –0.45). There was no linear relationship observed between *E. (A.) amstelodami* and *P. brevicompactum*. However, all other observed zero correlations were attributed to the three non-detect fungal species across all IMGOs in this study. These species included *A. sydowii*, *P. corylophilum*, and *P. spinulosum* that were not detected in any of the IMGOs. There were four different species that were negatively correlated with 18 or more species (i.e. at least 50% of the species measured by MSQPCR). For example, 30 of the 35 correlations associated with *Aspergillus unguis* ranged from –0.07 to –0.25.

Outdoor air

The concentration of species in outdoor air ranged from 0 SE m^{-3} (*Aspergillus ochraceus*, *A. sydowii*,

Figure 2. Correlogram of the 630 potential correlations of the 36 fungal species measured by MSQPCR in indoor air (a) and outdoor air (b). Correlation coefficients are shown both graphically and numerically with missing values indicating where species were not detected. Negative correlations are depicted in gradually darkening red and positive correlations are depicted by gradually darkening blue.

P. crustosum, *P. spinulosum*, and *P. variable*) to 10 859 (*W. sebi*) SE m^{-3} in terms of maximum counts (Table 1). The total concentration (based on non-geometric means) of fungal species in outdoor air was 4460 SE m^{-3} (range: 4–1158 SE m^{-3}), which is approximately 2× lower than the concentration observed in indoor air. The IQR of geometric means was 116 SE m^{-3} (25th and 75th percentiles: 31 and 147 SE m^{-3}). As observed in indoor air, there were select species that were observed only 1× across the study locations (e.g. *A. flavus*; see Table 1), as well as those species (n = 5) that were not detected [i.e. *A. ochraceus*, *A. sydowii*, *P. crustosum* (Group 2), *P. spinulosum*, and *P. variable*]. This is a similar trend of non-detect organisms as observed in the distribution of species in indoor air.

Similar to indoor air results, the outdoor air of each IMGO was represented by a distinctive pattern of fungal species. As shown in Fig. 1b, *W. sebi* was the dominant species in IMGO 1 at a concentration of 10 859 SE m^{-3}. A similar pattern for indoor air was also observed where *W. sebi* was most abundant; however, the concentration was two times lower in indoor air. Over 90% of the outdoor locations demonstrated a consistent presence of *C. herbarum*. Relatively high abundances of *C. herbarum* were observed in several IMGOs (e.g. IMGOs 5, 6, 11, and 20) and account for the majority of the fungal loading in these samples (ranging from ~30 to 6500 SE m^{-1}. The outdoor air was also predominantly loaded with *C. herbarum* in IMGOs 7 and 16 at concentrations of ~2400 and 3900 SE m^{-3}, respectively.

Figure 2. Continued.

Despite these relatively high concentrations, *E. nigrum* and *W. sebi* were present at even higher concentrations in IMGOs 7 and 16, respectively. For example, *E.nigrum* was 2× higher than *C. herbarum* in IMGO 7. These two species along with *Aspergillus penicilliodes* represent the predominant species present (i.e. of those tested). In IMGO 18, *E. (A.) amstelodami* was present at approximately 1230 SE m^{-3}, which was 5× higher than *C. herbarum* (250 SE m^{-3}), which further demonstrates that distinct patterns of fungal species were observed in outdoor air across the study locations.

In general, the correlations of fungal species from outdoor samples followed a similar pattern as indoor correlations (Fig. 2b). Of the 630 possible correlations, approximately 16% were moderately correlated and 28% were negatively correlated. All zero correlations (16%) were attributed to three non-detect fungal species: *A. sydowii*, *P. spinulosum*, and *P. variable*. A perfect correlation (i.e. $\rho = 1.0$) was observed between several *Aspergillus and Penicillium* genera: *A. versicolor* and *A. strictus*; *A. ochraceous* and *P. crustosum* (Group 2); *A. sclerotiorum* and *A. strictum*; and *P. chrysogenum* and *P. corylophilum*. There were five different species that were negatively correlated with 18 or more species (i.e. at least 50% of the species measured by MSQPCR). For example, 20 of the 35 correlations associated with *A. unguis* ranged from –0.05 to –0.20.

Indoor/outdoor air

To characterize the airborne burden of mold in IMGOs, indoor concentrations were compared with outdoor (I/O) concentrations. A total of 19 indoor–outdoor paired samples were collected for this comparison. In general, the median value of I/O ratios across all 36 species per

Table 2. Indoor–outdoor ratios for 36 fungal species across 19 paired samples and specific fungal species with a 10× shift in concentration by IMGO.

IMGO	Indoor[a]	Outdoor[a]	I/O	Fungal species with a 10× shift	
				Genus	Species
1	117	87.8	1.3	*Aspergillus*	*ochraceus*
					sclerotiorum
				Eurotium (A.)	*amstelodami*
				Penicillium	*purpurogenum*
				Stachybotrys	*chartarum*
4	13.5	4.0	8.2	*Aspergillus*	*niger*
					versicolor
				Eurotium (A.)	*amstelodami*
				Chaetomium	*globosum*
				Paecilomyce	*variotii*
				Penicillium	*brevicompactum*
					chyrsogenum
				Scopulariopsis	*brevicaulis*
				Trichoderm	*viride*
				Epicoccum	*nigrum*
				Mucor/Rhizopus	
5	22.1	6.1	14.3	*Aspergillus*	*fumigatus*
					niger
					ustus
				Eurotium (A.)	*amstelodami*
				Aureobasidium	*pullulans*
				Chaetomium	*globosum*
				Paecilomyces	*variotii*
				Penicillium	*brevicompactum*
					chyrsogenum
				Scopulariopsis	*chartarum*
				Trichoderma	*viride*
				Cladosporium	*cladosporiodes I*
				Epicoccum	*nigrum*
				Mucor/Rhizopus	
6	27.0	23.2	2.0	*Chaetomium*	*globosum*
				Paecilomyces	*variotii*
				Penicillium	*brevicompactum*
				Alternaria	*alternate*
				Mucor/Rhizopus	
7	310	120	1.1	*Trichoderma*	*viride*
10	50.1	36.9	1.4	*Aspergillus*	*unguis*
				Eurotium (A.)	*amstelodami*
				Aureobasidium	*pullulans*
				Paecilomyces	*variotii*
11	25.7	71.1	0.4	*Cladosporium*	*sphaerospermum*
13a	77.7	89.4	0.7	*Aspergillus*	*restrictus*
13b	172	89.4	0.9	*Penicillium*	*crustosum* (Group 2)
13c	537	89.4	4.1	*Scopulariopsis*	*brevicaulis*
14	155	224	0.5	*Eurotium (A.)*	*amstelodami*
				Stachybotrys	*chartarum*

Table 2. Continued

IMGO	Indoor[a]	Outdoor[a]	I/O	Fungal species with a 10× shift	
				Genus	Species
15	148	143	1.7	*Eurotium (A.)*	*amstelodami*
				Penicillium	*purpurogenum*
				Scopulariopsis	*brevicaulis*
				Trichoderma	*viride*
				Cladosporium	*cladosporiodes II*
				Mucor/Rhizopus	
				Rhizopus	*stolonifer*
16	83.6	182	0.4	*Aspergillus*	*penicilliodes*
				Rhizopus	*stolonifer*
17	102	54.1	1.0	*Paecilomyces*	*variotii*
				Wallemia	*sebi*
				Penicillium	*chyrsogenum*
18	576	46.6	1.7	*Aspergillus*	*fumigatus*
					versicolor
				Cladosporium	*sphaerospermum*
19	90.1	89.4	1.3	*Aspergillus*	*ochraceus*
					penicilliodes
					sclerotiorum
					versicolor
				Cladosporium	*sphaerospermum*
					cladosporiodes II
				Epicoccum	*nigrum*
20	97.9	93.2	1.4	*Aspergillus*	*restrictus*
				Chaetomium	*globosum*
				Scopulariopsis	*chartarum*
				Stachybotrys	*chartarum*
				Rhizopus	*stolonifer*
21	119	43.1	1.8	*Cladosporium*	*sphaerospermum*
				Wallemia	*sebi*
				Aspergillus	*ustus*
				Epicoccum	*nigrum*
				Mucor/Rhizopus	
22	34.6	3.5	0.2	*Aureobasidium*	*pullans*
				Scopulariopsis	*brevicaulis*
				Penicillium	*chyrsogenum*

[a]This value represents the geometric mean of all 36 species present at each IMGO with paired indoor–outdoor samples.

IMGO was 1.6 with a range of 0.4–6.2; almost 75% of these ratios were greater than 1 (Table 2). There were five operations with a ratio of less than 1 indicating that the concentration of outdoor species was higher than the concentration observed in indoor air. Overall, these results suggest the presence of indoor sources in a majority of these operations. Moreover, there were 29 different species where the geometric indoor concentrations were at least 10 times higher compared with outdoor concentrations, which indicates a prominent shift in fungal species. This 10 times shift in indoor concentrations typically ranged from 1 to 7 different species in a given operation (Table 2). However, there were two operations (IMGO #4 and #5) that had a 10× shift in 11 different species (30% of measured species) while a shift in 14 species was observed in IMGOs 5 and 13. While a shift was observed in allergenic species such as, *T. viride*, *Stachybotrys chartarum*, and *Aspergillus restrictus*, this shift also occurred in species that are commonly associated with outdoor sources, e.g. *E. nigrum*. Based on the

Table 3. Results from a two-sided paired Wilcoxon signed-rank test to evaluate differences in indoor and outdoor airborne fungal concentrations at each IMGO.

Fungal species	Indoor air	Outdoor air	*P*-values for Wilcoxon test statistic[a]
Aspergillus flavus	0.3	0.2	0.45
Aspergillus fumigatus	3.1	4.8	0.1
Aspergillus niger	19.7	19.4	0.06
Aspergillus ochraceus	0.4	0.0	0.49
Aspergillus penicillioides	2.4	6.7	0.22
Aspergillus restrictus	1.9	0.6	**0.0348**
Aspergillus sclerotiorum	3.3	0.6	0.18
Aspergillus sydowii	0.0	0.0	1
Aspergillus unguis	0.2	0.5	0.53
Aspergillus versicolor	1.6	0.4	0.57
Eurotium (A.) amstelodami	92.6	44.0	0.07
Aureobasidium pullulans	10.2	3.6	**0.02**
Chaetomium globosum	2.5	0.6	0.48
Cladosporium sphaerospermum	10.5	4.9	0.17
Paecilomyces variotii	9.5	2.9	0.116
Penicillium brevicompactum	4.1	1.3	0.077
Penicillium corylophilum	0.0	0.3	0.45
Penicillium crustosum (Group 2)	0.5	0.0	1
Penicillium purpurogenum	7.4	7.3	**0.0387**
Penicillium spinulosum	0.0	0.0	1
Penicillium variable	0.6	0.0	1
Scopulariopsis brevicaulis	3.6	2.3	**0.0378**
Scopulariopsis chartarum	8.4	6.9	**0.0364**
Stachybotrys chartarum	1.0	0.7	0.723
Trichoderma viride	2.0	1.1	0.723
Wallemia sebi	18.4	21.0	0.0921
Acremonium strictum	1.6	1.1	**0.0348**
Alternaria alternata	0.3	1.2	0.3159
Aspergillus ustus	9.6	8.9	**0.0393**
Cladosporium cladosporiodes I	19.6	44.0	**0.0403**
Cladosporium cladosporiodes II	3.1	1.8	0.1053
Cladosporium herbarum	204.6	234.7	**0.0343**
Epicoccum nigrum	66.9	52.3	**0.0378**
Mucor and Rhizopus group	2.7	0.6	0.4172
Penicillium chyrsogenum	0.8	0.3	0.1203
Rhizopus stolonifer	1.0	0.7	0.1278

[a]Those valued in bold indicate statistically significant differences.

Wilcoxon signed-rank test, almost 30% of the 36 species ($n = 10$) demonstrated a statistically significant difference ($P < 0.05$) between indoor and outdoor concentrations (Table 3). Five of these species are commonly associated with outdoor sources; however, only *C. cladosporiodes I* and *C. herbarum* were observed as more abundant outside than inside. The following species (including those with pathogenic and allergenic potential) were present at statistically significant higher concentrations inside the IMGOs than outside: *A. restrictus*, *Aureobasidium pullulans*, *P. purpurogenum*, *Scopulariopsis brevicaulis*, *S. chartarum*, *A. strictum*, *Aspergillus ustus*, and *E. nigrum*.

ERMI

An airborne ERMI score for indoor air in each IMGO was calculated using the aforementioned sum of logs equation (equation (1)) and are presented in Fig. 3. An average airborne ERMI score of 7 (range: –2.8 to 29.8) was determined for IMGOs in this study, indicating a

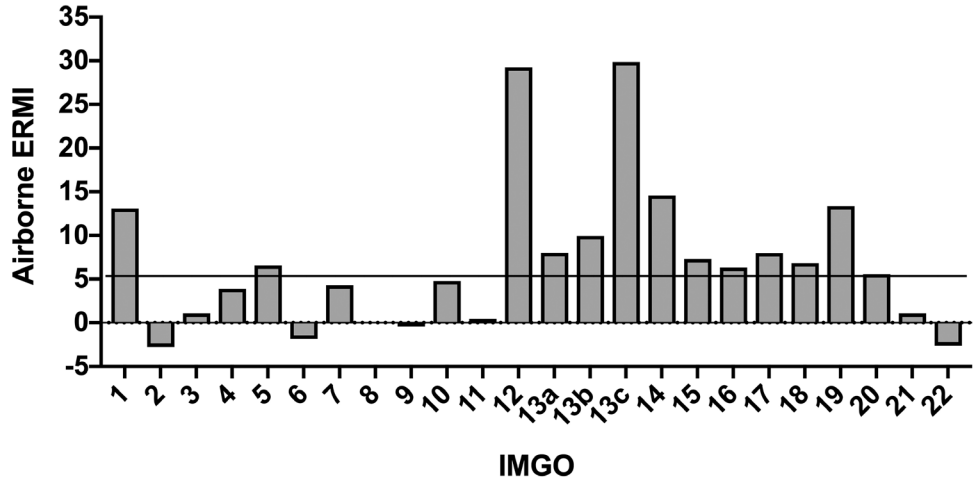

Figure 3. Airborne ERMI scores based on the presence of Groups 1 and 2 fungal species measured inside the grow room at each IMGO. High fungal burdens were determined for 19 of the IMGOs included in this study. The horizontal line represents an ERMI of 5, which has been demonstrated to be associated with asthma development and exacerbation. Only five of the IMGOs were represented by an ERMI score of less than 0 (indicating a low mold burden). The *y*-axis represents the calculated ERMI score for air samples and the *x*-axis is the IMGO. One operation (i.e. IMGO 13) contained three different units in a commercial office building.

relatively high burden of mold across a majority of operations. The IQR was 7.6 (0.9–8.5) further demonstrating a wide range of scores. There was a total of 13 IMGOs that were associated with an ERMI of 5 or higher, which has been demonstrated to be associated with asthma development and exacerbation (Kercsmar *et al.*, 2006; Reponen *et al.*, 2011). Of the 24 sampling locations, 5 of them had an ERMI score that was less than 0 (indicating a relatively low mold burden). The highest airborne ERMI score of 29 was associated with one of the units in IMGO 13 (commercial operation) while the highest ERMI score for residential operation was 13.

The ERMI scores inside the IMGOs were associated with the high concentrations of Group 1 species (i.e. those associated with water damage). The mean scores for Group 1 species were 15.8 with an IQR of 13 (Q1: 7.5; Q3: 20.4). The range of scores associated with Group 1 species extended from 2.4 to 46.4. Comparatively, the mean scores for Group 2 species were 8.9 (range: 1.9–17.2) with an IQR of 6 (6–12).

MSQPCR versus culture-based methods

A total of 228 culture-based air samples were collected concurrently with MSQPCR samples from across all operations. Data from these two methods were paired and evaluated to determine potential differences in the application of these methods. Results are based on detection of species between these two methods. Of the 36 species tested by MSQPCR, 14 species were not detected by culture-based methods. Based on McNemar's test, a range of concordant presence and absence were observed (Supplementary Table S3, available at *Annals of Work Exposures and Health* online). However, there were five species that were discordant at a statistically significant level ($P < 0.05$). These species include *A. versicolor*, *E. (A.) amstelodami*, *P. variotii*, *P. purpurogenum*, and *Penicillium chyrsogenum*. The species in bold text are those that demonstrated a higher probability of being detected by MSQPCR as compared with culture-based methods. Based on these findings, culture-based methods were further evaluated to determine if other species (not included in the MSQPCR panel) were relevant and specific to IMGOs and warrant integration into the MSQPCR/ERMI paradigm. There were 63 additional species identified in the culturable air samples colocated with the MSQPCR air samples (Supplementary Fig. S1, available at *Annals of Work Exposures and Health* online). A total of six additional fungal species that were measured in 20–24 of the sampling locations, which indicates a strong presence of non-ERMI species in the IMGOs. However, four of these taxa were only classified down to the genus level: *Penicillium* sp., *Cladosporium* sp., *Aspergillus* sp., and *Alternaria* sp. While specific species were not identified, the remaining two taxa were classified as Sterile (White) and yeast.

To evaluate the applicability of the ERMI to air samples, vacuum samples inside the grow rooms were collected since the ERMI was developed for settled dust (i.e. 5 mg of dust). Only four vacuum samples were collected in conjunction with the air samples due to extenuating circumstances (e.g. concrete floors in the grow room,

which did not yield 5 mg of dust). Vacuum samples were collected from adjacent rooms when possible (data not shown). Further, every sampling campaign was coordinated with the North Metro Drug Taskforce, which limited sampling time in any given operation. As such, the scope of this study was focused on deploying and rotating the air samplers through different locations. The geometric mean of the total dust concentration was 1117 SE mg^{-1} of dust (Table 1). The geometric means in these samples ranged from 1.0 to 547.2 SE mg^{-1} of dust with *A. pullulans* having the highest geometric mean. Comparing air to dust MSQPCR samples, over 50% of the species detected in the air were present in higher concentrations. However, there were no statistically significant differences between these two matrices (air and dust) were observed based on the Wilcoxon signed-rank test (Supplementary Table S2, available at *Annals of Work Exposures and Health* online). Given the small sample size, additional comparisons and research are warranted to further evaluate the applicability of using the ERMI for air samples.

Discussion

Given the environmental conditions (e.g. temperature and humidity) necessary for mass cultivation of cannabis, we hypothesized that IMGOs foster high concentrations of airborne fungi. These exposures are of particular concern in this nascent industry due to previous occupational health research in agricultural settings that links fungal exposure to adverse respiratory health outcomes (Schaeffer *et al.*, 2017). The need to improve monitoring and implement rapid diagnostics for identification of fungal burden extends beyond health and safety in the workplace. For example, the Denver Department of Health and Environment identified increased levels of mold and yeast in a marijuana grow operation that lead to a voluntary recall in retail products that may be considered unsafe for the consumer (Nicholson, 2019).

In this study, we evaluated the use of MSQPCR and ERMI as potentially highly sensitive and rapid diagnostic tools for quantitative measurement of fungal species in the indoor air of marijuana grow operations. Our results suggest that workers in IMGOs are likely exposed to elevated concentrations of airborne fungi that are associated with allergenic, pathogenic, and toxigenic potential. Allergic respiratory diseases, especially asthma, have been associated with sensitization to a wide range of fungal species, including those species related to the *Alternaria*, *Penicillium*, *Aspergillus* and *Cladosporium* genera (Heseltine *et al.*, 2009). All of these taxa were present in IMGOs at varying concentrations. Further, over 80% of the species had geometric indoor concentrations at least 10× higher compared with outdoor concentrations, which indicates a prominent shift in fungal species in the indoor environment.

This is the first study to characterize the atmospheric fungal burden in IMGOs using MSQPCR methods and an airborne ERMI scale. The ERMI score has been used to describe fungal burden of indoor environments in several epidemiological studies. One study found that the ERMI score was significantly higher in a school with high prevalence of asthma as compared with a school with low prevalence of asthma (Vesper *et al.*, 2015). Two other studies found that ERMI scores were significantly higher in the homes of children with asthma (Vesper *et al.*, 2008, 2013). A review of six epidemiological studies found that the ERMI score may serve as a useful tool to link mold contamination to some asthma health effects (Vesper and Wymer, 2016). These studies relied on dust samples and were focused on residential, school, or office environments. Our findings indicate that IMGOs had ERMI scores that were higher than 75% of the residential homes used to create the index (only the top 25% of homes had ERMI scores ≥5). In comparison, approximately 60% of IMGOs evaluated had an ERMI scores of 5 or higher. These scores maybe driven by high concentrations of select ERMI species found in the IMGOs. Specifically, *T. viride* had a max count of >64 000 SE m^{-3} as compared with those concentrations (geometric mean: 1602 SE g^{-1}) observed in settled dust of water-damaged homes of asthmatic children. This species was present at significantly higher concentrations in homes of people with asthma as compared with controls as previously described in Vesper *et al.* (2013). In terms of magnitude, these results confirmed our hypothesis that IMGOs have a relatively high burden of fungal exposure.

The ERMI was originally developed for the measurement of fungal contamination that accumulated over time in settled dust. Due to concerns with inhalation hazards present during work shifts, we evaluated the applicability of using the ERMI scale to describe the burden of airborne fungi. No statistically significant differences were observed in the concentrations of species between collocated dust and air samples collected inside the grow rooms in this study. Méheust *et al.* (2013) presented the first use of the ERMI scale to describe the airborne loading of fungal contamination inside homes located in the northwest region of France. They found significant correlations of fungal species between air and dust samples. Based on our collective work, the ERMI may be applied to characterize the fungal burden in air

samples in addition to settled dust samples. Given our limited number of collocated samples in air and settled dust, additional evaluations are needed within these indoor environments to further demonstrate associations between sampling matrices.

In addition to comparison of the sampling matrices for fungal species, our overarching research question sought to understand if the ERMI scale based on MSQPCR was superior to traditional culture-based methods for fungal sampling in the context of occupational settings characterized by high potential for fungal growth. The limitations of traditional mold sampling methods are well documented, and include lack of standardized methods, issues with reproducibility and selectivity, and limited sampling windows that do not reflect the dynamic nature of spore abundance(Godish and Godish, 2007; Crawford *et al.*, 2009; Pityn and Anderson, 2013). In the context of IMGOs, the MSQPCR method had higher odds of detecting several species included in the ERMI compared with culture-based methods. The MSQPCR method detected a total of 14 species (almost 50% of the fungal species included in the panel) that were not detected by the culture-based methods. Further, statistically significant differences were observed between MSQPCR and culture methods for the detection of five fungal species; of these, MSQPCR was more likely to detect three species: *E. (A.) amstelodami*, *P. variotii*, and *P. purpurogenum*; all of which are Group 1 species that may play an important role in exposure–health outcomes. In summary, MSQPCR is better suited for detecting a majority of the species used to calculate the ERMI.

Despite the above observations, the MSQPCR demonstrated limitations compared with culture-based methods. Specifically, there were 63 additional species identified in the culturable air samples colocated with the MSQPCR air samples; six of these species were measured in nearly all of IMGOs. As ERMI was based on fungal species found in flood-damaged households, this panel of fungal species may not be optimal for IMGOs. The strong presence of these other airborne species not included in the ERMI indicates that a clear need remains to elucidate an IMGO-specific ERMI to ensure that relevant species (with capacity to elicit adverse health outcomes) are included. In accordance with these findings, we suggest that although the MSQPCR method for fungal detection may have several advantages compared with culture-based methods, the ERMI scale based on MSQPCR may not be an appropriate measurement tool for IMGOs until a catalog of fungal species relevant to the industry is identified. The correlations and presence and absence of species will help inform future studies focused on developing an IMGO-specific index that protects against adverse respiratory outcomes among these workers.

There are additional limitations to application of the ERMI method in occupational settings such as IMGOs. The method is based upon a specific geographical region and sample subset (Meklin *et al.*, 2007, Vesper *et al.*, 2007). An ERMI score of 0 is associated with the 50th percentile of homes included in the original study where the algorithm was developed (Vesper *et al.*, 2009). The median ERMI scores of 6.0 across IMGOs are considerably higher than the home median concentrations. Also, given that outside airborne ERMI scores in our analysis were higher than 75% of homes reported in the ERMI method, region and season may have a large impact on Group 2 species. Other limitations in this evaluation were a small sample size and sampling time (i.e. 1 h). While MSQPCR-based sampling methods for airborne fungi were 30× longer as compared with the culture-based method, important health-related taxa may have been missed given the dynamic nature of fungi. Longer sampling periods that span a full work shift may provide more relevant and reliable estimates of fungal species. Further, previous research suggesting an index score of 5 or higher is associated with asthma development and exacerbation was largely based on outcomes of children living in water-damaged homes. There is still a need to elucidate a relevant score for workplace conditions and health effects of workers.

Our evaluation of the ERMI based on MSQPCR as a rapid diagnostic and risk assessment tool for industrial hygienists in the IMGO setting is equivocal. When relying solely on the ERMI score to evaluate the fungal burden in IMGOs, it is possible to miss high concentrations of specific fungal species that may be of concern. It is important to consider the Groups 1 and 2 values individually and to identify specific species driving these scores. Further, despite high concentrations of an individual or combination of Group 1 species present in an environment, this signal may be attenuated by comparably high concentrations of Group 2 species. Consequently, a relatively low ERMI score may be calculated; however, workers may be still at risk. Therefore, it is critical for practitioners to ascertain the presence and abundance of specific fungal taxa measured by MSQPCR to inform control strategies, especially for those individuals susceptible to such exposures. Future studies will be essential for further verifying the suitability of an airborne ERMI and the catalog of fungal species included in Groups 1 and 2. It is important to note that this study was conducted on clandestine IMGOs prior to the legalization of recreational cannabis in Colorado in 2014.

As such, ventilation rates in these operations may not have been acceptable for occupants. The air quality in these IMGOs was most likely compromised or modified to limit detection by law enforcement. These conditions may not translate to current ventilation practices found in legally operating settings in the present. However, to our knowledge, there are no ventilation standards for IMGOs at this time. Developing a tool that can serve as a rapid diagnostic for fungal concentrations, especially for species that may be an inhalation hazard for exposed workers, should be a priority for industrial hygiene as well as the sector.

Conclusions

Fungal contamination (especially allergenic and toxigenic taxa) and attendant exposures are a major concern among workers in IMGOs. Results of this study indicated that MSQPCR can be used to characterize the fungal burden present in IMGOs and that a predominant shift in species was observed from outdoor to indoor samples. Moreover, some of the reported levels may even be considered extreme in comparison to residential measurements. The ERMI method did have greater odds of detecting certain species than traditional viable methods and airborne ERMI scores were higher than those observed in studies of other environments where upper respiratory effects were reported. However, ERMI did not identify all relevant fungal species associated with this specific occupational environment. We identified several issues with using the ERMI calculation. At this time, the catalog of fungal species needs to be optimized for the occupational setting to ensure adequate coverage, especially for those species expected to be found in this burgeoning industry. Further research is necessary to elucidate the link between the ERMI score of airborne samples, worker exposure and health effects in grows to generate an acceptable index score for use in occupational exposure assessments.

Supplementary Data

Supplementary data are available at *Annals of Work Exposures and Health* online.

Results from Wilcoxon signed-rank test to evaluate the differences between concentrations of fungal species identified in indoor and outdoor samples, and in air versus dust samples; results from McNemar's test to evaluate the agreement between MSQPCR and culture-based methods in the detection of fungal species; and frequency of detection of non-ERMI species from culture-based methods.

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Conflict of Interest

The authors declare no conflict of interest relating to the material presented in this article. Its contents, including any opinions and/or conclusions expressed, are solely those of the authors.

References

Blanc PD, Quinlan PJ, Katz PP *et al.* (2013) Higher environmental relative moldiness index values measured in homes of adults with asthma, rhinitis, or both conditions. *Environ Res*; **122**: 98–101.

Crawford C, Reponen T, Lee T *et al.* (2009) Temporal and spatial variation of indoor and outdoor airborne fungal spores, pollen, and (1→3)-β-D-glucan. *Aerobiologia (Bologna)*; **25**: 147–58.

Douwes J, Thorne P, Pearce N *et al.* (2003) Bioaerosol health effects and exposure assessment: progress and prospects. *Ann Occup Hyg*; **47**: 187–200.

Fierer N, Liu Z, Rodriguez-Hernandez M *et al.* (2008) Short-term temporal variability in airborne bacterial and fungal populations. *Appl Environ Microbiol*; **74**: 200.

Ghosh TS, Vigil DI, Maffey A *et al.* (2017) Lessons learned after three years of legalized, recreational marijuana: the Colorado experience. *Prev Med*; **104**: 4–6.

Godish D, Godish T. (2007) Total airborne mold particle sampling: evaluation of sample collection, preparation and counting procedures, and collection devices. *J Occup Environ Hyg*; **5**: 100–6.

Governing. (2018) State Marijuana Laws in 2018 Map [WWW Document]. Governing. Available at http://www.governing.com/gov-data/safety-justice/state-marijuana-laws-map-medical-recreational.html. Accessed 29 April 2019.

Haugland R, Vesper S. (2002) *Method of identifying and quantifying specific fungi and bacteria*. US Pat. 6,387,652.

Heseltine E, Rosen J, World Health Organization. (2009) *WHO guidelines for indoor air quality: dampness and mould*. Copenhagen: WHO.

Institute of Medicine (US) Committee on Damp Indoor Spaces and Health. (2004) *Damp indoor spaces and health*. Washington, DC: National Academies Press.

Johnson LI, Miller JD. (2012) Consequences of large-scale production of marijuana in residential buildings. *Indoor Built Environ*; **21**: 595–600.

Kercsmar CM, Dearborn DG, Schluchter MD *et al*. (2006) Reduction in asthma morbidity in children as a result of home remediation aimed at moisture sources. *Environ Health Perspect*; **114**: 1574–80.

Maier SL, Mannes S, Koppenhofer EL. (2017) The implications of marijuana decriminalization and legalization on crime in the United States. *Contemp Drug Probl*; **44**: 125–46.

Martyny JW, Serrano KA, Schaeffer JW *et al*. (2013) Potential exposures associated with indoor marijuana growing operations. *J Occup Environ Hyg*; **10**: 622–39.

Méheust D, Le Cann P, Reponen T *et al*. (2013) Possible application of the Environmental Relative Moldiness Index in France: A pilot study in Brittany. *Int J Hyg Environ Health*; **216**: 333–340.

Meklin T, Haugland RA, Reponen T *et al*. (2004) Quantitative PCR analysis of house dust can reveal abnormal mold conditions. *J Environ Monit*; **6**: 615–20.

Meklin T, Reponen T, McKinstry C *et al*. (2007) Comparison of mold concentrations quantified by MSQPCR in indoor and outdoor air sampled simultaneously. *Sci Total Environ*; **382**: 130–4.

Nicholson K. (2019) High mold and yeast levels prompt recall of statewide marijuana products over health concerns [WWW Document]. *Denver Post*. Available at https://www.denverpost.com/2019/10/14/denver-marijuana-recall/. Accessed 11 April 2020.

Niemeier RT, Sivasubramani SK, Reponen T *et al*. (2006) Assessment of fungal contamination in moldy homes: comparison of different methods. *J Occup Environ Hyg*; **3**: 262–73.

Orens A, Light M, Lewandowski B *et al*. (2018) Market size and demand for Marijuana in Colorado 2017 market update. Available at https://www.colorado.gov/pacific/sites/default/files/MED%20Demand%20and%20Market%20%20Study%20%20082018.pdf. Accessed 6 June 2020.

Pityn PJ, Anderson J. (2013) Air sampling of mold spores by slit impactors: yield comparison. *J Environ Sci Health A Tox Hazard Subst Environ Eng*; **48**: 1485–90.

Priyamvada H, Singh RK, Akila M *et al*. (2017) Seasonal variation of the dominant allergenic fungal aerosols—one year study from southern Indian region. *Sci Rep*; **7**: 11171.

Reponen T, Vesper S, Levin L *et al*. (2011) High Environmental Relative Moldiness Index during infancy as predictor of age seven asthma. *Ann Allergy Asthma Immunol*; **107**: 120–6.

Schaeffer JW, Reynolds S, Magzamen S *et al*. (2017) Size, composition, and source profiles of inhalable bioaerosols from Colorado dairies. *Environ Sci Technol*; **51**: 6430–40.

Spicer R, Gangloff H. (2007) Establishing site specific reference levels for fungi in outdoor air for building evaluation. *J Occup Environ Hyg*; **2**: 257–66.

Van Dyke MV, Martyny JW, Marola J *et al*. (2012) Efficacy of occupant-collected dust samples in the evaluation of residential allergen and fungal levels. *J Occup Environ Hyg*; **9**: 14–24.

Vesper S, Barnes C, Ciaccio CE *et al*. (2013) Higher Environmental Relative Moldiness Index (ERMI) values measured in homes of asthmatic children in Boston, Kansas City, and San Diego. *J Asthma*; **50**: 155–61.

Vesper S, Choi H, Perzanowski MS *et al*. (2016) Mold populations and dust mite allergen concentrations in house dust samples from across Puerto Rico. *Int J Environ Health Res*; **26**: 198–207.

Vesper S, McKinstry C, Cox D *et al*. (2009) Correlation between ERMI values and other moisture and mold assessments of homes in the American Healthy Homes Survey. *J Urban Health*; **86**: 850–60.

Vesper S, McKinstry C, Haugland R *et al*. (2007) Development of an environmental relative moldiness index for US homes. *J Occup Environ Med*; **49**: 829–33.

Vesper S, McKinstry C, Haugland R *et al*. (2008) Higher Environmental Relative Moldiness Index (ERMIsm) values measured in Detroit homes of severely asthmatic children. *Sci Total Environ*; **394**: 192–6.

Vesper S, Prill R, Wymer L *et al*. (2015) Mold contamination in schools with either high or low prevalence of asthma. *Pediatr Allergy Immunol*; **26**: 49–53.

Vesper S, Wymer L. (2016) The relationship between environmental relative moldiness index values and asthma. *Int J Hyg Environ Health*; **219**: 233–8.